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Activity of antioxidant enzymes in different pathophysiological states

Aktivita antioxidačních enzymů za různých patofyziologických stavů

Doctoral Thesis

Supervisor: RNDr. Eva Tvrzická, CSc.

Prague, 2013

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VÁVROVÁ, Lucie: Activity of antioxidant enzymes in different pathophysiological states. [Aktivita antioxidačních enzymů za různých patofyziologických stavů]. Praha, 2013. 152s, 7 příloh. Disertační práce. Univerzita Karlova v Praze, 1. Lékařská fakulta, IV. Interní klinika; Vedoucí závěrečné práce/Školitel: RNDr. Eva Tvrzická, CSc.

Acknowledgment

I would like to thank all who supported me. First of all I would like to thank Prof. MUDr. Aleš Žák, DrSc., who enabled to curry out all the clinical studies and helped me during writing doctoral thesis. Thanks belong to my supervisor RNDr. Eva Tvrzická, CSc.

I would like to thank all my colleagues from Lipid Laboratory of Institute of Clinical Biochemistry and Laboratory Diagnostics, first of all Mgr. Jana Kodydková and then Mgr. Barbora Staňková and RNDr. Marek Vecka, Ph.D. and technician Jiřina Trávníčková, Iva Smítalová and Růžena Marshová.

I also appreciated very much the help from my clinical colleagues from different units of 4th Department of Internal Medicine namely Doc. MUDr. Miroslav Zeman, CSc., MUDr. František Novák, Ph.D., MUDr. Jaroslav Macášek, and MUDr. Tomáš Krechler, CSc. I am very grateful to the research nurse Jitka Bartíková for the screening and precise data collection.

I also thank very much to my parents for their support and patience during my long days and nights lived out with the work on my thesis.

The studies presented in this thesis were supported by the Czech Ministry of Health research grants: IGA MZ NR/8943 – 4; IGA MZ NS 9769-4; IGA NR 8806-3 and by the research project MSM0021620820 of Ministry of Education, Youth and Sports, Czech Republic.

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Abstract

Background: Oxidative stress is supposed to be implicated in the pathogenesis of several diseases which are connected with increased formation of reactive oxygen and nitrogen species (RONS). Oxidative stress could play an important role in the pathogenesis of inflammation and sepsis, acute and chronic pancreatitis or in the development of cancer. Organisms are protected against RONS from antioxidant system that is composed of antioxidant enzymes and non-enzymatic antioxidants. To the most important antioxidant enzymes belong superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase, glutathione reductase and paraoxonase (PON). The aim of this Doctoral Thesis was to investigate the behaviour of three of these antioxidant enzymes – CuZnSOD, CAT and PON1 in different pathophysiological states.

Materials and methods: The activities of CuZnSOD, CAT and PON1 were measured in six different pathophysiological states. Forty patients with metabolic syndrome (MetS), 35 women with depressive disorder (DD), 30 septic patients (SP), 50 patients with pancreatic cancer (PC), 50 patients with chronic pancreatitis (CP) and 13 patients with acute pancreatitis (AP) were included in different studies together with sex- and age-matched healthy controls (CON). Patients with AP and SP were observed in the course of the disease and samples were taken four times (three times, respectively). The enzymatic activities were determined with spectrophotometric kinetic methods. In all these studies also the levels of oxidative stress markers were measured.

Results: The activity of CAT was found to be decreased in patients with sepsis or septic shock, MetS and PC in comparison with CON, while in patients with DD, CP and AP no differences in CAT activity were detected. The activities of CuZnSOD were in the contrast to CAT either increased or unaffected. Increased activities of CuZnSOD were observed in MetS, DD, PC and SP, while no differences in CuZnSOD activities were found between CP or AP and CON. In all observed pathophysiological states the arylesterase activity of PON1 was measured and was found to be decreased (with the exception of DD) in comparison with CON.

Conclusion: It was shown, that all selected diseases are connected with increased oxidative stress, which leads to the changes in antioxidant enzymes activities.

Abstrakt

Úvod: U onemocnění spojených se zvýšenou tvorbou reaktivních sloučenin kyslíku a dusíku (RONS) se přepokládá účast oxidačního stresu v jejich patogenezi. Oxidační stres hraje roli v řadě onemocnění; významnou úlohu má v patogeneze zánětu a sepse, akutní a chronické pankreatitidy či vzniku rakovinného bujení. Organismus je proti působení RONS chráněn antioxidačním systémem, který je tvořen antioxidačními enzymy a neenzymatickými antioxidanty. K nejdůležitějším antioxidačním enzymům se řadí superoxiddismutasa (SOD), katalasa (CAT), glutathionperoxidasa (GPX), glutathionreduktasa (GR) a paraoxonasa (PON). Cílem této disertační práce bylo vyšetření aktivity tří těchto antioxidačních enzymů – SOD, CAT, PON za různých patofyziologických stavů.

Materiál a metody: U šesti různých patofyziologických stavů byly měřeny aktivity CuZnSOD, CAT a PON1. Do jednotlivých studií bylo zařazeno 40 pacientů s metabolickým syndromem (MetS), 35 žen s depresivní poruchou (DD), 30 pacientů se sepsí (SP), 50 pacientů s karcinomem pankreatu (PC), 50 pacientů s chronickou pankreatitidou (CP) a 13 pacientů s akutní pankreatitidou (AP). Ke každé sledované skupině pacientů byla zařazena též kontrolní skupina spárovaná na základě věku a pohlaví (CON). Pacienti s AP a SP byli sledováni v průběhu jejich onemocnění a vzorky byly nabírány celkem 4x respektive 3x. Aktivity antioxidačních enzymů byly stanovovány spektrofotometrickými kinetickými metodami. Ve všech studiích byly zároveň měřeny markery oxidačního stresu.

Výsledky: Snížené hladiny aktivit CAT byly pozorovány u pacientů se sepsí či septickými šokem, MetS a PC v porovnání s kontrolami, zatímco u pacientů s DD, CP a AP nebyly zjištěny žádné rozdíly v aktivitě CAT při srovnání s CON. U pacientů s MetS, DD, PC a SP byly zjištěny zvýšené aktivity CuZnSOD, i když u pacientů s CP a AP nebyly pozorovány rozdíly v aktivitě CuZnSOD při srovnání s CON. U všech sledovaných patofyziologických stavů (s výjimkou depresivních poruch) byly nalezeny snížené aktivity PON1 v porovnání s CON.

Závěr: Provedené studie ukazují, že všechna sledovaná onemocnění jsou spojena se zvýšeným oxidačním stresem, v rámci kterého dochází k ovlivnění chování námi sledovaných antioxidačních enzymů.

Abbreviations

Å	Ångström (dimension 10^{-10}m)
ANOVA	analysis of variance – statistical models
AP	acute pancreatitis
APACHE II	score - Acute Physiology and Chronic Health Evaluation II
Apo	apolipoprotein
ARDS	acute respiratory distress syndrome
CAD	coronary artery disease
CAT	catalase
CD-LDL	conjugated dienes in precipitated LDL
CON	“healthy” controls
CP	chronic pancreatitis
CRP	C-reactive protein
CuZnSOD	CuZn-superoxide dismutase
DD	depressive disorder
DSM	Diagnostic and Statistical Manual of Mental Disorders
EDTA	ethylenediaminetetraacetic acid
ELISA	Enzyme Linked-Immuno-Sorbent Assay
GPX	glutathione peroxidase
GR	glutathione reductase
GSH	reduced glutathione
H	helix
HAM-D	Hamilton Depression Rating Scale
Hb	hemoglobin
HDL	high density lipoprotein
IL	interleukin
LDL	low density lipoprotein
MetS	metabolic syndrome
NAD	nicotine amid adenine dinucleotide
NADP	nicotine amid adenine dinucleotide phosphate
NBT	nitro blue tetrazolium salt
NT	nitrotyrosine
Ox-LDL	oxidized-LDL

PC	pancreatic cancer
PCT	prokalcitonin
PON1	paraoxonase 1
PON1-A	arylesterase activity of PON1
PON1-L	lactonase activity of PON1
PON1-P	paraoxonase activity of PON1
RONS	reactive oxygen and nitrogen species
ROS	reactive oxygen species
SP	septic patients
S1	1st sampling of sepsis
S7	2nd sampling of sepsis (7 days after onset of sepsis)
R7	3rd sampling of septic patients (7 days after recovery from sepsis)
SAA	serum amyloid A
S.D.	standard deviation
TC	total cholesterol
TG	triglycerides
TNF	tumour necrosis factor
TRIS	2-Amino-2-hydroxymethyl-propane-1, 3-diol

1. Introduction

Antioxidants are compounds that control the redox-balance in biological systems. Even in small concentrations they prevent the oxidation of instable substrates and/or eliminate the reactive oxygen and nitrogen species (RONS). Antioxidants represent heterogeneous group of chemical compounds with respect to their structure and function. They could be divided according to their location to extracellular (paraoxonase, albumin, bilirubin, etc.), membrane (vitamin E, phospholipases, β-carotene) and intracellular (catalase, glutathione, ascorbic acid, etc.) antioxidants and according to their character to enzymatic and non-enzymatic antioxidants. To the most important antioxidant enzymes belong superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR) and paraoxonase (PON). The most important non-enzymatic antioxidant is reduced glutathione (GSH).

In physiological conditions there is equilibrium between RONS's creation and degradation in organism due to the antioxidants. In the course of metabolic processes different RONS are created and organism is able to utilize them; for example the elimination of pathogenic organisms by leukocytes or nitrogen oxide as neurotransmitter. However, overproduction of RONS in connection with impaired function of antioxidant system leads to oxidative stress. It was shown that RONS and/or oxidative stress could be involved in the pathogenesis of some diseases such as atherosclerosis, diabetes mellitus, inflammation and sepsis or neurodegenerative diseases. The role of RONS in clinical medicine was in detail described by Macasek et al. (2011; supplement 5).

The research of RONS and antioxidants became actual theme in medicine in the recent years. This doctoral thesis deals with the activity of three antioxidant enzymes – SOD, CAT and PON in different pathophysiological states. Activities of these enzymes together with levels of oxidative stress markers were established in patients with metabolic syndrome (MetS), depressive disorder (DD), sepsis or septic shock (SP), pancreatic cancer (PC), chronic (CP) and acute pancreatitis (AP).

1.1. Catalase

Catalases (CAT, H_2O_2 : H_2O_2 – oxidoreductase, EC: 1.11.1.6), enzymes with long history, that goes back to the 19th century, when they became one of the first sources of valuable information about the nature and behaviour of enzymes (Zámocký & Koller, 1999). The name catalase became in 1900 according to its catalytic action on hydrogen peroxide (Loew, 1900). Human catalase belongs to the group of monofunctional hem-containing catalases; members of this large subgroup are found in almost all aerobically respiring organisms (Chelikani et al., 2004; Zámocký & Koller, 1999). Catalase is primarily an intracellular enzyme; the highest concentrations in mammalians are in erythrocytes and liver and occasionally in kidney (Deisseroth & Dounce, 1970). In tissues such as liver, catalase is found predominantly in peroxisomes (Quan et al., 1986). In the following text, human catalase will be discussed.

1.1.1. Structure

Human catalase (Figure 1a) is a tetrameric protein of 244kDa comprising four identical subunits of 69.7 kDa. Each subunit contains 527 amino acid residues, one heme group with iron in the ferric state and a tightly bound molecule of NADPH (Kirkman & Gaetani, 1984; Ko et al., 2000; Safo et al., 2001). The subunit could be conceptually divided into four domains (Figure 1b): N-terminal threading arm (residues 5-70), wrapping loop, β-barrel comprised of two four-stranded sheets and α-helical domain composed of four helices α4 to α7 (residues 155-207) and four C- terminal helices α16 to α19 (residues 440-501). The human catalase is extensively hydrated, only the hydrophobic β-barrel and the immediate vicinity of the active site are substantially devoid of the structural water molecules (Putnam et al., 2000).

The active site of enzyme is internally located and contains the heme prosthetic group, namely iron(III) protoporphyrin IX (Kirkman & Gaetani, 2006). The structure of CAT shows, that the iron protoporphyrin is pentacoordinated (Figure 1c); to the substrate accessible at his distal side. The reactivity of the heme is tuned by electron donation by the Tyr358 ligand, and neutralization of the carboxylate charge by Arg72, Arg112 and Arg365. In the active site charge relay are involved four amino acid residues on the proximal side of the heme: His218, Asp348, Arg354 and Tyr358 (Putnam et al., 2000). There are three channels that have been implicated as potentially having a role in access

to the active site – the perpendicular = main channel (Figure 1d), the lateral = minor channel and channel leading from heme to the central cavity of the tetramer. All three channels are quite narrow; this restricts accessibility to relatively small molecules. (Switala & Loewen, 2002).

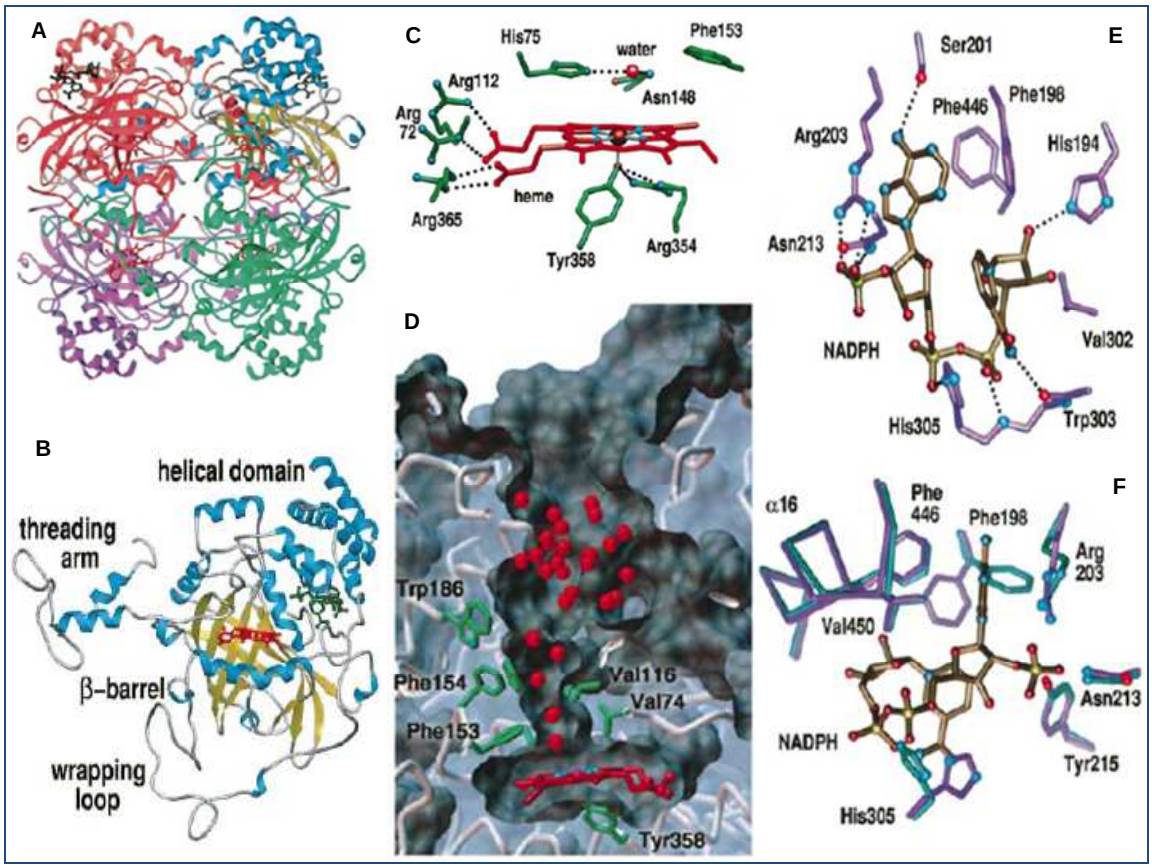


Figure 1: Structure of human catalase

A: Structure of human catalase; B: individual unit of human CAT, helices – blue, β-barrel - yellow; C: Active site of human catalase; D: main channel; E, F: NADPH binding site, NADPH - bronze; heme – red; (Putnam et al., 2000).

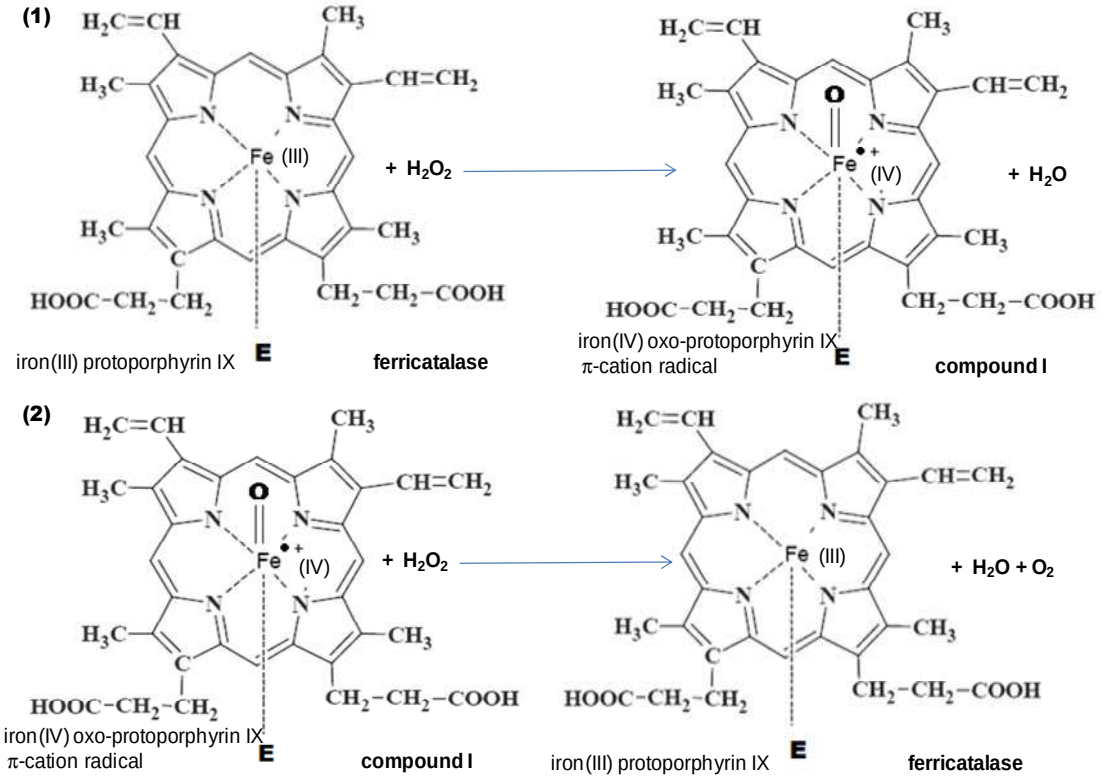
Human catalase binds the molecules of NADPH on the surface of the molecule at a cleft between the α6 and α7 helices of helical domain and the β-barrel. The NADPH binding pockets (Figure 1e, f) contain His194, Arg203, Val302, Trp303 and His305, which may be considered to be signatures for NADPH binding (Putnam et al., 2000; Chelikani et al., 2004). The reduced form of NADP - NADPH has the highest affinity to catalase surface. The order of affinity is NADPH > NADH >> $NADP^+$ > NAD^+. The dissociation constant for NADPH is less than 10nM (Kirkman & Gaetani, 1984).

Normal erythrocytes contain 1.31-2.71µg CAT/mg Hb. These values correspond to an expected concentration of 6.6 to 13.7 µM for catalase-bound NADPH in human erythrocytes (Kirkman & Gaetani, 1984). The concentration of unbound NADP in human erythrocytes is approximately 2µM (Kirkman et al., 1987).

Human erythrocyte catalase has been crystallized in orthorhombic, monoclinic, tetragonal and hexagonal unit cells till now (Maté et al., 1999; Ko et al., 2000; Putnam et al., 2000; Safo et al., 2001).

1.1.2. Function

The main function of catalase is the decomposition of hydrogen peroxide to water and oxygen – catalatic activity. It is generally accepted, that this reaction occurs in two steps.



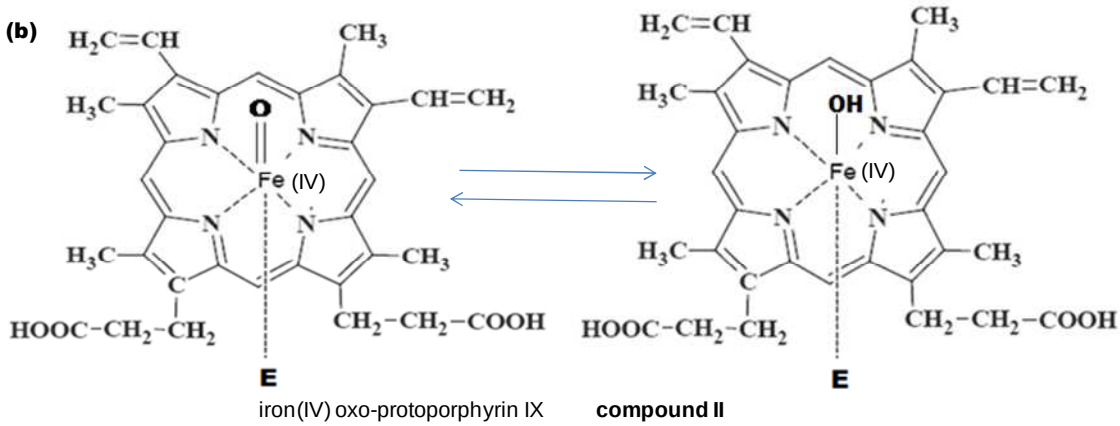
Scheme I: Catalatic reaction of catalase

In the first step reacts one molecule of hydrogen peroxide with the iron(III) protoporphyrin IX group of CAT (ferricatalase). In this step compound-I and one molecule of water are formed, through what is known as heterolytic scission of peroxide

O-O bond. Compound-I is iron(IV) oxo-protoporphyrin π-cation radical of CAT. In the second step compound-I reacts with the second molecule of hydrogen peroxide, a molecule of oxygen and molecule of water are produced and the iron(III) protoporphyrin IX group of CAT is regenerated (Scheme I). Both forms of CAT – ferricatalase and compound-I – are active forms of this enzyme (Kirkman et al., 1987; Almarsson et al., 1993; Kirkman & Gaetani, 2006).

In the reaction of compound I with a single electron of the compound-II, the inactive form of CAT, is formed. Compound-II is iron(IV) oxo-protoporphyrin IX (Kirkman & Gaetani, 2006), however it was provided that it exists also in iron(IV) hydroxy-protoporphyrin IX form (Scheme II), (Rovira, 2005).

(a) Compound I + e^- → Compound II



Scheme II: Formation of compound II

During lengthy exposure of CAT to H_2O_2, the CAT bound NADPH became oxidized to $NADP^+$ and the activity of enzyme fell to about one-third of the initial value. NADPH protects CAT against inactivation by H_2O_2 (Kirkman & Gaetani, 1987). The exact role and function of NADPH in CAT was discussed in different articles (Kirkman & Gaetani, 1987; Hillar & Nichols, 1992; Almarsson et al., 1993; Olson & Bruice, 1995; Kirkman et al., 1999; Rovira 2005; Kirkman & Gaetani, 2006). It was estimated, that NADPH prevents the formation of compound II and also mildly increases the rate of removal of compound II (Kirkman et al., 1999). Almarson et al. (1993) proposed that NADPH could react with both - compound I and compound II (Scheme III).

Reaction with compound I

1. Compound I + NADPH $\rightarrow$ Compound II + $NADPH^{+\cdot}$
2. $NADPH^{+\cdot}$ + B $\rightarrow$ $NADP^{\cdot}$ + BH^{+}
3. Compound II + $NADP^{\cdot}$ $\rightarrow$ ferricatalase + $NADP^{+}$

Reaction with compound II

1. Compound II + NADPH $\rightarrow$ ferricatalase + $NADPH^{+\cdot}$
2. $NADPH^{+\cdot}$ + B $\rightarrow$ $NADP^{\cdot}$ + BH^{+}
3. $NADP^{\cdot}$ + O_2 $\rightarrow$ $NADP^{+}$ + $O_2^{-\cdot}$

Scheme III: Reactions of NADPH with compound I and II

In addition to a very efficient catalatic reaction mode, catalase could also catalyse 2-electron peroxidations of short-chain aliphatic alcohols at reasonable rates (Zámocký & Koller, 1999). The catalatic reaction predominates when the H_2O_2 concentration is higher than 10^{-4}M, while below this concentration in the presence of an acceptable hydrogen donor the peroxidatic reaction dominates (Maté et al., 1999). Peroxidatic activity is relatively slow (Kirkman & Gaetani, 2006). There are three families of enzymes which could remove hydrogen peroxides *in vivo*: catalases, glutathione peroxidases and peroxiredoxins. Catalase may be the key enzyme for H_2O_2 removal in peroxisomes, although peroxiredoxin 5 may contribute. At low H_2O_2 concentrations GPX1 and peroxiredoxins are responsible for its degradation. Although peroxiredoxins are slower at catalysing H_2O_2 degradation than GPX1, it is suggested that at low H_2O_2 concentrations, peroxiredoxins dispose most of H_2O_2 generated inside the cells (Halliwell & Gutteridge, 2007).

In 2003 Heck et al. discovered, that in keratinocytes CAT could generate reactive oxygen species (ROS) in response to UV light. The ability of the enzyme to generate ROS depended on the dose of UV light utilized and on the concentration of CAT (Heck et al., 2003). Chelikani et al. speculate that the NADPH cofactor could have a role in ROS generation suggesting another role for NADPH in catalase physiology (Chelikani et al., 2004).

1.1.3. Determination

For the determination of the activity of CAT numerous methods were used. The first methods were based on permanganate (von Euler & Josephson, 1927) or iodimetric (Yamagata et al., 1952) titrations. For the reaction with permanganate, the photometric detection was later applied (Goldblith & Proctor, 1950). In 1958 Dobkin and Glantz presented colorimetric method for CAT determination based on reaction of hydrogen peroxide with ferricyanide in alkaline solution (Dobkin & Glantz, 1958). Another possibility of colorimetric determination of the residual hydrogen peroxide after incubation with CAT was described by Cohen et al. (1996). In this method ferrous ions and thiocyanate were used for the H_2O_2 determination (Cohen et al., 1996)

The flotation rate of a paper disk saturated with the enzyme solution was also utilized for the CAT activity measurement. The method is based on the liberation of oxygen due to the action of catalase on hydrogen peroxide (Gagnon et al., 1959; Lamoureux et al., 1987). Wheeler et al. (1990) have automated the CAT activity determination that was previously described by Johansson & Borg (1988). The method measured peroxidatic activity of CAT and is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of hydrogen peroxide. The formaldehyde produced is then measured spectrophotometrically with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) as a chromogen (Wheeler et al., 1990; Johansson & Borg, 1988). In 1996 discontinuous measurement of CAT activity at nearly physiological levels of hydrogen peroxide was described by Ou & Wolff. They used ferrous oxidation in xylenol orange for CAT activity determination (Ou & Wolff, 1996).

For the determination of CAT activity also polarographic (Góth & Mészáros, 1975), gasometric (Siqueira et al., 1999), fluorometric (Wu et al., 2003) and chemiluminiscence (Mueller et al., 1997) assays were utilized. However, the most used method is those of Aebi; it is spectrophotometric method, based on the measurement of decomposition of hydrogen peroxide at 240nm (Aebi, 1974).

Concentration of CAT was determined with immunochemical assays (Higashi et al., 1961). The polarography was used as other possibility to measure the CAT concentration. In this method CAT was measured simultaneously with SOD (Rigo & Rotilio, 1977).

1.2. Superoxide dismutase

In 1969 McCord and Fridovich have shown that copper proteins that were previously isolated from bovine (in 1939, "hemocuprein") and human erythrocytes ("erythrocuprein") are associated with the enzymatic activity of superoxide free radical anion dismutation. The proteins became its name according to its function – superoxide dismutase (McCord & Fridovich, 1969).

In mammalians three different isoforms of superoxide dismutase have been biochemically and molecularly characterized. Firstly, SOD1, or CuZnSOD (EC 1.15.1.1) that is a copper and zinc-containing homodimer found almost exclusively in intracellular cytoplasmic space. The second form of SOD containing manganese as cofactor is SOD2, or MnSOD (EC 1.15.1.1). MnSOD is initially synthesized in cytosol as a tetramer containing a leader peptide which targets enzyme exclusively to the mitochondrial spaces. SOD3, or EC-SOD (EC 1.15.1.1) exists as a copper and zinc-containing tetramer with a signal peptide that directs this enzyme exclusively to the extracellular space (Zelko et al., 2002).

This Thesis is focused on the human CuZnSOD that is widely distributed in the nucleus and cytosol of human cells and in lower concentration also in peroxisomes. (Crapo et al., 1992).

1.2.1. Structure

Human CuZnSOD is a dimeric enzyme of relative molecular mass 32,000 Daltons, containing two identical subunits of circa 150 amino acids. Each monomer is built upon a β-barrel motif and possesses two large functionally important loops, called the electrostatic and zinc loops, which encase the metal-binding region that binds one copper and one zinc ion (Figure 2a). It has been found that in the active site the copper ion is coordinated by the nitrogen atoms of four histidine residues (His46, His 48, His 63 and His120) and by a water molecule. One of the histidine residues (His63) bridges copper and zinc when the copper ion is in the 2^+ oxidation state (Figure 2b). Coordination of zinc is completed by two other histidine residues (His71 and His80) and one aspartate residue (Asp83) in a tetrahedral geometry (Figure 2c); (Potter et al., 2006; Hart et al., 1998; Banci et al., 1999). A hydrogen bond network further stabilizes the structure around the metal ions (Valentine et al., 2005).

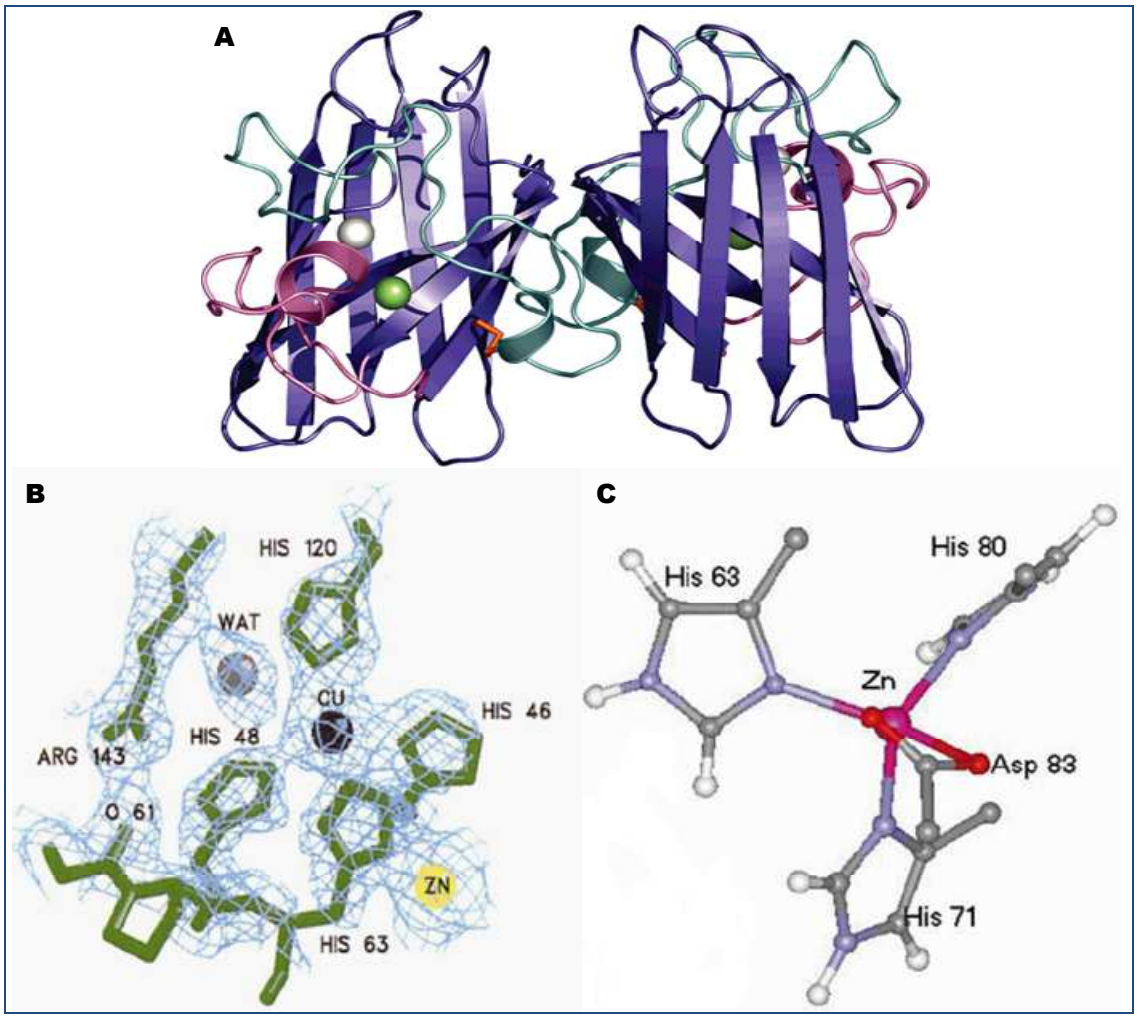


Figure 2: Structure of human CuZn-Superoxide dismutase

A: The structure of CuZnSOD dimer, copper ions: green, zinc ions: gray, the intramolecular disulfide bonds: orange, zinc loops: cyan, electrostatic loops: pink (Potter et al., 2006); B: Active center of CuZnSOD, Wat: water (Hart et al., 1998); C: The coordination of zinc in CuZnSOD (Banci et al., 1999).

1.2.2. Function

The main function of superoxide dismutase is the decomposition of superoxide to hydrogen peroxide and oxygen - dismutation. It is widely accepted, that this reaction occurs in two steps. The first step of the reaction (Scheme IV) involves the reduction of Cu(II) by superoxide, producing dioxygen and Cu(I) form of SOD. In the second step Cu(I) form of SOD is oxidized by another superoxide, producing hydrogen peroxide (Rotilio et al., 1972a; Rotilio et al., 1972b; Klug-Roth et al., 1973; Fielden et al., 1974).

(1) $O_2^{-\cdot}$ + Cu(II)ZnSOD $\Rightarrow$ O_2 + Cu(I)ZnSOD

(2) $O_2^{-\cdot} + Cu(I)ZnSOD + 2H^+ \Rightarrow Cu(II)ZnSOD + H_2O_2$

Scheme IV: Reaction mechanism of superoxide dismutase

The reduced Cu(I) form of the enzyme has another structure than the oxidized one: the copper ion undergoes a shift in position, moving away from the His63 nitrogen where was bound in the oxidized form of the enzyme. The copper ion also releases the water ligand, going from irregular five-coordinate geometry to a nearly trigonal planar three-coordinate configuration. His63 is bind exclusively to the zinc ion that remains in tetrahedral geometry (Figure 3); (Valentine et al., 2005).

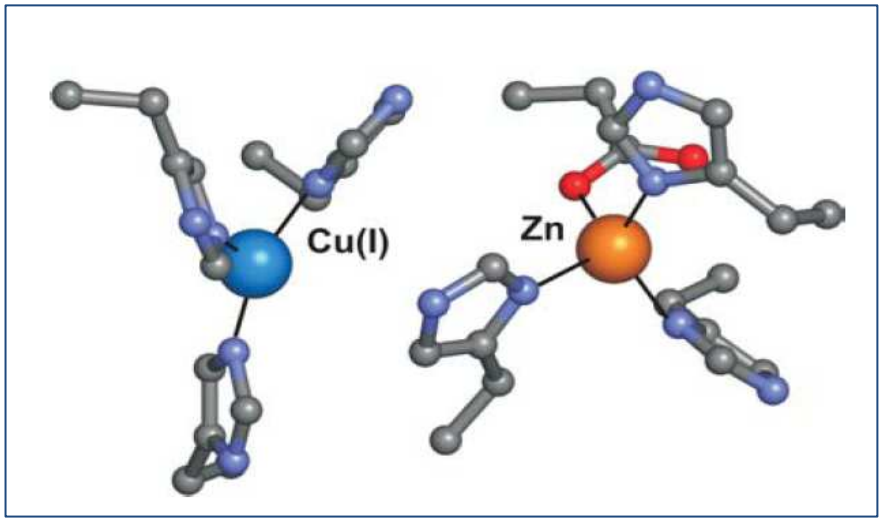


Figure 3: Active site of CuZn-Superoxide dismutase in reduced form (Valentine et al., 2005)

1.2.3. Determination

Indirect two steps methods are mainly used for the determination of CuZnSOD activity, because of the low stability of its substrate – superoxide (Flohé & Ötting, 1984). In the first step of the reaction superoxide is generated either enzymatically or non-enzymatically. To give a controlled rate of superoxide generation, the system of xanthine plus xanthine oxidase is used (McCord & Fridovich, 1969; Beauchamp & Fridovich, 1971; Štípek et al. 1995). Superoxide could be generated non-enzymatically by the autooxidation of epinefrin (Misra & Fridovich, 1972) or pyrogallol (Marklund & Marklund, 1974), or by the degradation of potassium superoxide (Marklund, 1976). In the second step of the reaction the generation of superoxide is spectrophotometrically detected using indicator which reacts with superoxide. Nitro blue tetrazolium salt - NBT

(Beauchamp & Fridovich, 1971; Štípek et al., 1995) or cytochrome c (McCord & Fridovich, 1969) could be used as indicators.

1.3. Paraoxonase

Paraoxonase gene family is composed of three members PON1, PON2 and PON3 that are located adjacently on chromosome 7 in humans (Précourt et al., 2011). The PON proteins share 60% sequence identity. The name, paraoxonase, is purely historical, as the PON members are a hydrolase family with one of the broadest specificities known (Harel et al., 2004). This thesis is focused on the human PON1.

Paraoxonase-1 (PON1; EC 3.1.8.1.) is synthesized in the liver and secreted into the blood, where it associates with HDL (high-density lipoprotein), (Deakin & James, 2004).

1.3.1. Structure

Human paraoxonase 1 is glycoprotein with the size of 43 000 kDa, including 354 amino acids and four potential *N*-linked glycosylation sites (Josse et al., 2001). The molecule of PON1 forms six-bladed β-propeller (Figure 4A, 4B), where each blade contains four strands (Harel et al., 2004). A disulfide bond at Cys 41–Cys 352 forms the covalent closure of the N and C termini which is conserved throughout the PON family (Josse et al., 2001).

In the central tunnel of the β-propeller, there are two calcium ions 7.4 Å apart from each other. One calcium ion could be found at the top (Ca1) and one in the central section (Ca2) of the tunnel (Harel et al., 2004; Harel et al., 2007). Ca2 (Figure 4C) is most probably a “structural calcium” whose dissociation leads to irreversible denaturation of protein (Kuo & La Du, 1998). Ca1 is assigned as the “catalytic calcium” and it seems to interact with five protein residues (the side chain oxygens of Asn224, Asn270, Asn168, Asp269 and Glu53) one water molecule and one of the oxygens of a phosphate ion (Figure 4D); (Harel et al., 2004).

There are four potential *N*-glycosylation sites on PON1: two glycan chains are linked to asparagine residues in positions 253 and 324 on surface loops (Kuo & La Du, 1995), two other potential positions are Asn224 and Asn270 in the central tunnel of the propeller and are largely inaccessible (Harel et al., 2004). Glycosylation is not essential

for the hydrolytic activities of PONs (Josse et al., 1999), however may be important in increasing their solubility and stability, or in preventing nonspecific binding to the cell membranes (Harel et al., 2004).

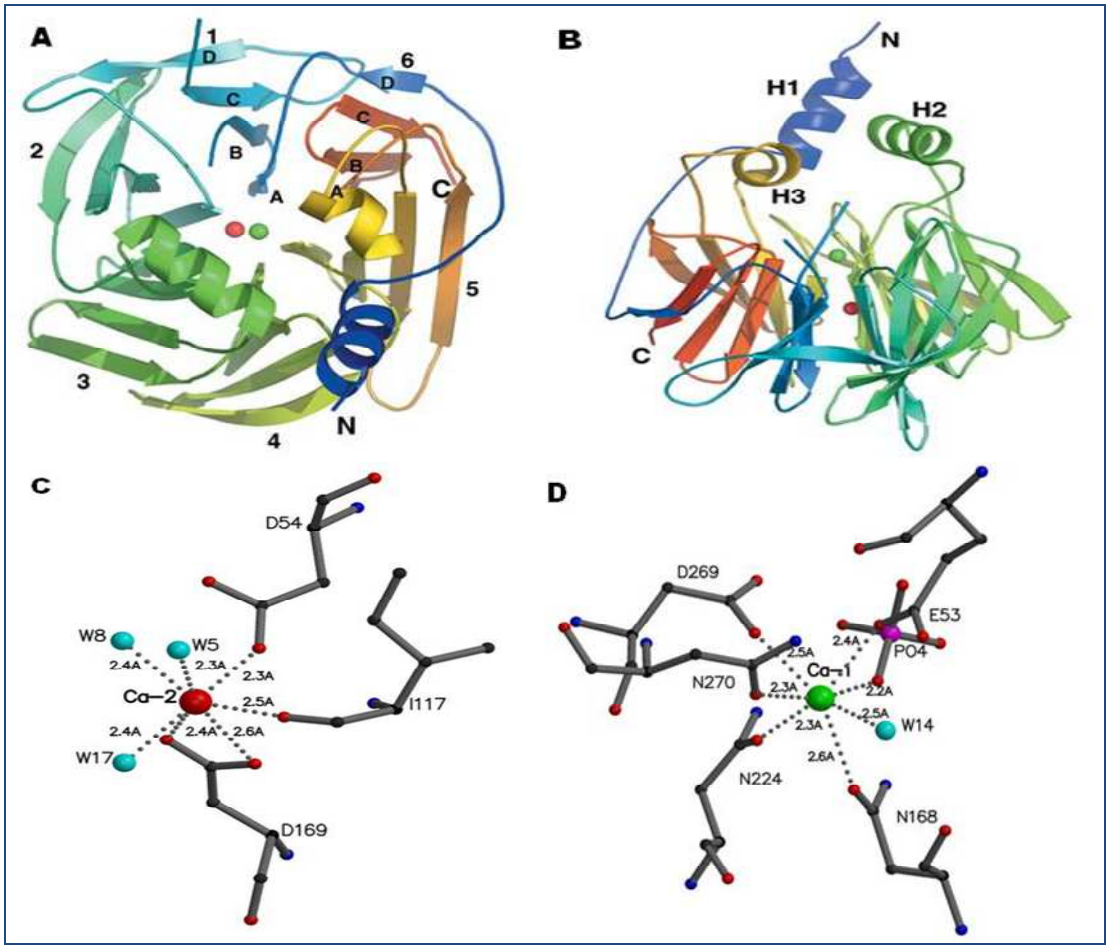


Figure 4: Structure of paraoxonase 1

A: Overall structure of PON1, view of the six-bladed β-propeller from above. Shown are the N and C termini, and the two calcium atoms in the central tunnel of the propeller (Ca1, green; Ca2, red); B: Overall structure of PON1, a side view of the propeller, including the three helices at the top of the propeller (H1–H3); C: Detailed view of PON1's calcium binding site for the inner (structural) calcium (Ca-2). D: Detailed view of PON1's calcium binding site for the upper (catalytic) calcium (Ca-1); W: water molecule; N: Asn, E: Glu, D: Asp; (Harel et al., 2004).

PON1 is synthesized in the liver and secreted into the blood, where associates with HDL particles. In anchoring of PON1 to HDL, the hydrophobic N terminus of PON1 is thought to be involved (Sorenson et al., 1999). The entire sequence of the N terminus is compatible with a transmembrane helix and forms two helixes: H1 hydrophilic part of N

terminus and H2 with amphipathic character. Helices H1 and H2 form two adjacent hydrophobic patches that provide a potential membrane-binding surface (Figure 5). The interface with HDL was further defined by a characteristic 'aromatic belt' rich in tryptophan and tyrosine side chains (Tyr185, Phe 186, Tyr190, Trp194, Trp202) and by a lysine (Lys 21) side chain on H1 (Harel et al., 2004).

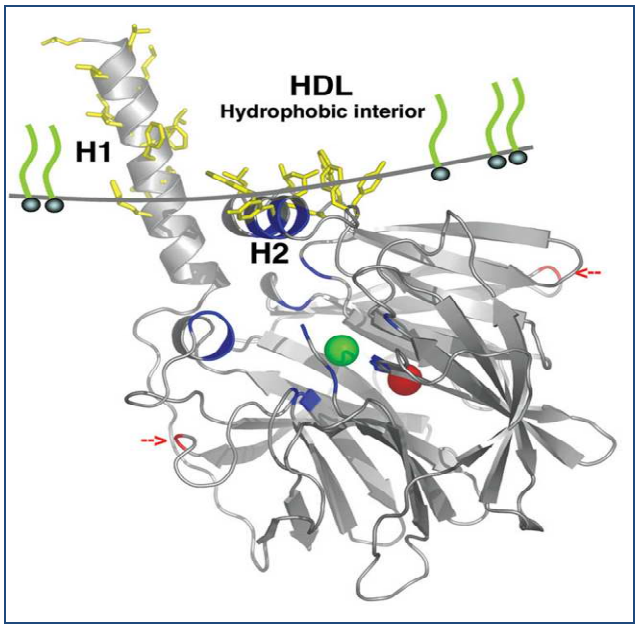


Figure 5: Association of paraoxonase 1 with HDL particles

Proposed model for anchoring of PON1 to the surface of HDL. Hydrophobic residues proposed to be involved in HDL anchoring (side chains yellow). The active site and the selectivity-determining residues are blue, and the proposed glycosylation sites (Asn253 and Asn324) are red; (Harel et al., 2004).

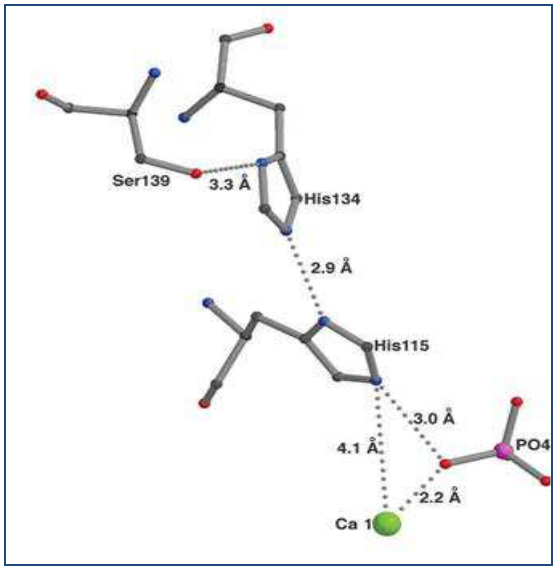
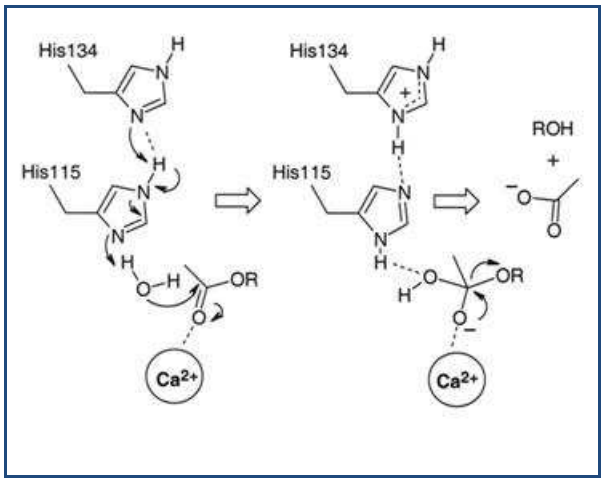


Figure 6: The postulated catalytic site of paraoxonase 1 (Harel et al., 2004)

At the very bottom of the active site cavity lays the upper calcium (Ca1), and a phosphate ion. The study of Josse et al. (2001) identified four histidine residues (His115, His134, His243, His285) and one tryptophan residue (W281) to be essential for the activity of PON1. Later in the study of Harel et al. (2004) the His115-His134 dyad was identified (Figure 6) and it was hypothesized that His115 (4.1 Å from Ca1) acts as a general base to deprotonate a single water molecule and generate the attacking hydroxide, whereas His134 acts in a proton shuttle mechanism to increase His115's basicity (Harel et al., 2004).

1.3.2. Function

A variety of physiological roles have been proposed for PONs. Serum PON1 catalyzes the hydrolysis and thereby the inactivation of oxons like paraoxon (from which it takes its name; diethyl 4-nitrophenyl phosphate), chlorpyrifos oxon and diazoxon which are toxic metabolites of organophosphate insecticides like parathion (*O,O*-Diethyl *O*-(4-nitrophenyl) phosphorothioate) and chlorpyriphos (O,O-Diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate). Paraoxonase is able to hydrolyze also the nerve agents sarin ((RS)-propan-2-yl methylphosphonofluoridate) and soman (3,3-dimethylbutan-2-yl methylphosphonofluoridate). In addition, PON1 hydrolyzes arylesters like phenylacetate, thiophenylacetate and 2-naphthylacetate (Davies et al., 1996; Aviram et al., 1998a). It also hydrolyses different aromatic and aliphatic lactones as well as cyclic carbonates like homogentisic acid lactone, dihydrocoumarin, γ -butyrolactone and homocysteine thiolactone (Billecke et al., 2000; Rajkovic et al., 2011).



Scheme V: Reaction mechanism of paraoxonase 1 (Harel et al., 2004).

Proposed mechanism of action of PON1 on ester substrates such as phenyl and 2-naphthylacetate is shown in the scheme V. The first step involves deprotonation of a water molecule by the His-His dyad to generate hydroxide anion that attacks the ester carbonyl producing an oxyanionic tetrahedral intermediate. This intermediate breaks down (second step) to an acetate ion and either phenol or 2-naphthol (Harel et al., 2004).

It was hypothesises (Draganov et al., 2005; Nguyen et al., 2009) that the native activity of PON1 could be lactonase activity, for which the free cystein 284 in PON1 structure is very important (Yilmaz, 2012) and that the physiological substrate could be some derivates of fatty acid oxidation process such as 5-hydroxy - 6E, 8Z, 11Z, 14Z - eicosatetraenoic acid (5-HETE) lactone that resides in HDL or some lactones which are consumed as food ingredients or drug metabolites (statins, spironolactone and glucocorticoid y-lactones). Furthermore was shown that homocysteine thiolactone is naturally occurring substrate of PON1 (Jakubowski, 2000). Homocysteine thiolactone is a metabolite of homocysteine that can impair protein function leading to endothelial dysfunction and vascular damage (Macharia et al., 2012). By detoxifying homocysteine thiolactone, PON1 protects proteins against homocysteinylation (Perla-Kaján & Jakubowski, 2010; Yilmaz, 2012). Other group of lactones that could be degraded by PON1 are acyl-homoserine lactones, small quorum sensing signaling molecules of Gram-negative bacterium Pseudomonas aeruginosa. Pseudomonas aeruginosa uses these lactones to coordinate phenotypic changes, including biofilm formation and virulence factor secretion (Estin et al., 2010; Rai, 2012).

In vitro assays have shown that PON1 can inhibit LDL (low-density lipoprotein) lipid peroxidation and inactivate LDL-derived oxidized phospholipids. This could potentially reduce the serum content of the oxidized lipids involved in the initiation of atherosclerosis (Mackness et al., 1991; Mackness et al., 1993; Watson et al., 1995).

It was shown, that PON1 have also peroxidase-like activity (Aviram et al., 1998b; Précourt et al., 2011) – it is capable to hydrolyze hydrogen peroxide, reduce lipoprotein peroxides (by 19%) and cholesteryl linoleate hydroperoxides (by 90%).

1.3.3. Determination

The activity of paraoxonase 1 is determined towards different types of substrates. For the measurement of arylesterase activity of PON1 phenyl acetate as substrate is used (Eckerson et al., 1983). The paraoxonase activity of PON1 is determined using paraoxon as substrate (Eckerson et al., 1983; Hasselwander et al., 1998; Ferré et al., 2002). For the determination of PON1 activity towards lactones different substrates are used – methods with 5-thiobutyl butyrolactone (Gaidukov &Tawfik, 2007), dihydrocoumarin (Draganov et al., 2000), 2-coumaranone (Billecke et al., 2000) and homogentistic acid lactone (Billecke et al., 2000) were previously described.

2. Aims and scopes

The aim of this doctoral thesis was to investigate the behaviour of three antioxidant enzymes – superoxide dismutase, catalase and paraoxonase in different pathophysiological states.

This thesis was focused on antioxidant enzymes activities and their changes in acute phase of the disease such as sepsis or acute pancreatitis, where the activities were followed up in the course of sepsis or acute pancreatitis.

In chronic states such as metabolic syndrome, pancreatic carcinoma or chronic pancreatitis the antioxidant enzymes activities were compared with those of healthy volunteers.

3. Materials and Methods

3.1. Pathophysiological states studied

Antioxidant status and oxidative stress markers were observed in different types of pathophysiological states. Patients with metabolic syndrome (MetS), depressive disorder (DD), sepsis (SP), pancreatic cancer (PC), chronic pancreatitis (CP) and with acute pancreatitis (AP) were involved in the studies of antioxidant status. The short characteristics of all studied groups are summarized in the Table 1. Informed consent was obtained from all participants. The study protocols were approved by the Ethical Committee of the First Faculty of Medicine, Charles University Prague or by the Ethical Committee of the University Teaching Hospital, Prague.

Table 1: Characteristics of the studied groups of patients

	N (M/F)	Age (years)	Reference
MetS	40 (20/20)	58 (53 - 62)	Vavrova et al. (2013) – Supplement 1
DD	35 (0/35)	61.5 ± 16.5	Kodydkova et al. (2009) – Supplement 3
SP	30 (16/14) 19 (10/9)	57.7 ± 15.3 74 (56-79)	Novak et al. (2010) – Supplement 4 Vavrova et al. (under review) – Supplement 7
PC	50 (40/10)	63 (56 - 68)	Kodydkova et al. (2013) – Supplement 2
CP	50 (40/10)	59 (53 - 65)	
AP	13 (9/4)	56.1 ± 21.5	Vavrova et al. (2012) – Supplement 5

MetS: metabolic syndrome, DD: depressive disorder, SP: patients with sepsis, PC: pancreatic carcinoma, CP: chronic pancreatitis, AP: acute pancreatitis; M: male, F: female

The exclusion criteria in all studies were the following: current antioxidant therapy, excessive alcohol consumption (> 30 g/day), hormonal replacement therapy, supplementation with polyunsaturated fatty acids; chronic, immunosuppressive and anti-inflammatory therapy; manifestation of cardiovascular and/or cerebrovascular

diseases, diabetes mellitus type 1, liver (with exception of non-alcoholic fatty liver disease) and kidney diseases (creatinine > 130 μmol/l), microalbuminuria (urinary albumin 30 - 300 mg/day), hypothyroidism and recent infections, malignancies (with exception of PC). Persons who were operated on upper gastrointestinal tract (in the previous 1 year) and subjects after systemic inflammation in the previous 6 months were also excluded.

3.2. Blood sample collection and preparation

All blood samples were obtained after overnight fasting. Blood was taken puncturing a peripheral vein. Activities of CAT and CuZnSOD were measured in haemolysed erythrocytes and both activities of PON1 were determined in serum. Serum was also used for the determination of all other parameters. The blood samples for CAT and CuZnSOD measurements were collected into the tubes with K_2EDTA. Erythrocytes were separated by the centrifugation at 3500 rpm at 4°C for 10 minutes and then washed three times with a NaCl isotonic solution (9 g/l). Serum was prepared following coagulation in vacutainer tubes, by centrifugation at 3500 rpm at 4°C for 10 min. The samples were stored at -80°C until assay. The haematological parameters were carried out by routine laboratory techniques using an autoanalyzer (Coulter LH750 - haematological analyzer, Beckman Coulter).

3.3. Measurements of antioxidant enzyme activities

The methods for measurement of antioxidant enzymes: catalase, superoxide dismutase and arylesterase – PON1 activities were described in detail in the publication of Kodydkova et al. (2009). The CAT activity was determined by the modified method of Aebi (1974). The determination is based on the monitoring of the rate of H_2O_2 degradation at 240 nm. The reaction mixture in cuvettes contained 876 μl of 50 mM potassium phosphate buffer, pH = 7.2 and 25 μl of diluted sample. The reaction was started after 10 minutes of incubation at 30 ºC by addition of 99 μl of 10 mM H_2O_2. Blank was run for each sample. Catalase activity was calculated using the molar extinction coefficient of H_2O_2 43.6 M^{-1} cm^{-1} and expressed as kU/g haemoglobin (U = μmol/min).

The activity of CuZnSOD was determined according to the modified method of Štípek et al. (1995). The reaction mixture in cuvettes contained 700 μl of 50 mM potassium

phosphate buffer, pH = 7.2; 50 µl of xanthine oxidase; 100 µl of NBT and 50 µl of diluted sample. The reaction was started after 10 minutes of incubation at 25 ºC by addition of 100 µl of 1 mM xanthine. The rate of NBT-formazan generation was monitored spectrophotometrically at 540 nm. Blank was run for each sample. Superoxide dismutase activity was calculated by means of calibration curve and expressed as U/g haemoglobin (U = µmol/min).

The arylesterase activity of PON1 was measured according to the method of Eckerson et al. (1983) using phenylacetate as a substrate. Briefly, 900 µl of 20 mM Tris-HCl buffer containing 1 mM $CaCl_2$, pH=8.0 was added to cuvettes followed by 50 µl of diluted serum sample. The reaction was started by addition of 50 µl of 100 mM phenylacetate. The rate of phenol generation was monitored spectrophotometrically at 270 nm. Blank was run for each sample. Arylesterase activity of PON1 was calculated using the molar extinction coefficient of the produced phenol, 1310 $M^{-1}cm^{-1}$ and expressed as U/ml serum (U = µmol/min).

Paraoxonase activity of PON1 was measured using paraoxon (O,O-Diethyl O-(4-nitrophenyl) phosphate) as a substrate in tubes containing 940 µl of 90 mM Tris-buffer (pH = 8.5, with 2 mM $CaCl_2$) and 50 µl of 100 mM paraoxon. The reaction was started by addition of 10 µl of serum and measured at 405 nm at 25 °C. The activity was calculated using the molar extinction coefficient of the produced p-nitro-phenol, 18053 $M^{-1}cm^{-1}$ and expressed in U/l serum.

The lactonase activity of PON1 was determined according to the modified method of Draganov et al. (2000). Briefly, to 800 µl of 50 mM TRIS-buffer (pH = 8, with 1 mM $CaCl_2$) in cuvettes 100 µl of diluted serum sample was added. The reaction was started after incubation at 30 °C for 5 minutes with 100 µl of dihydrocoumarin (final concentration 1 mM). The increase in absorbance at 270 nm was monitored along 2 minutes. Kinetic rate was estimated during the linear phase of reaction and converted to the enzyme activity using the molar extinction coefficient of the reaction product 3-(2-hydroxy-phenyl)-propionate (ε = 1295 $dm^3mol^{-1}cm^{-1}$), (Rainwater, 2009). The lactonase activity of PON1 was expressed as U/ml serum (U = µmol/min).

3.4. Measurements of markers of oxidative stress

In all studies concentrations of conjugated dienes in precipitated LDL (CD-LDL) as marker of oxidative stress were measured. In some studies also levels of nitrotyrosine (NT) and/or oxidized LDL (ox-LDL) were established. Concentrations of CD-LDL were measured by the modified method of Wieland et al. (1983). Serum low density lipoproteins were isolated by the precipitation method of Ahotupa et al. (1996). 110 μl of serum with EDTA (10:1) was added to 1 ml of 0.064 M citrate buffer (pH = 5.05, with 50.000 U/l heparin), the suspension was then incubated for 10 min at room temperature. The precipitated lipoproteins were separated by centrifugation at 2800 rpm for 10 min and the pellet was resuspended in 100 μl of NaCl isotonic solution (9g/l). Lipids were extracted by dichlormethan – methanol (2:1) mixture, for the phase separation 250 μl redistilled water was used. The mixture was centrifuged at 3000 rpm for 5 min. The 800 μl of lower layer (infranatant) was dried under nitrogen, redissolved in 300 μl of cyclohexane, and analyzed spectrophotometrically at 234 nm. The concentration of CD was calculated using the molar extinction coefficient (2.95×10^4 $M^{-1}cm^{-1}$) and expressed as mmol/l serum.

The concentration of NT was measured using the ELISA kit (Hycult biotech, HK 501). The NT ELISA is a solid-phase enzyme-linked immunosorbent assay based on the sandwich principle. The levels of ox-LDL were established with the ELISA kit (Mercodia). Oxidized LDL ELISA is a solid phase two-site enzyme immunoassay. This method is based on the mouse monoclonal antibody, which is directed against a conformational epitope in oxidized ApoB-100.

3.5. Measurements of antioxidant enzyme cofactors and lipid parameters

The concentrations of serum amyloid A (SAA) were determined using solid phase sandwich ELISA kit (Invitrogen, KHA 0011).

All routine clinical tests were measured at the Institute for Clinical Biochemistry and Laboratory Diagnostics of General University Hospital in Prague: Copper and zinc were measured using atomic absorption spectrometry. Concentration of calcium was established by the photometric method with o-kresolftalexon. High-density lipoprotein

cholesterol was determined in the supernatant after precipitation of lipoproteins B by PTA/Mg^{2+}, using the kit (Boehringer Mannheim, Germany). Concentrations of apo A-I were measured by the Laurell rocket electroimmunoassay using standard and specific antibodies (Behringwerke Marburg, Germany).

3.6. Statistical analysis

The mean ± standard deviation (S.D.) for parametric values and median (25^{th}-75^{th} percentiles) for non-parametric values were used for the data expression. Normality of distribution of data was tested with Shapiro-Wilks W test. Differences between two compared groups were tested with t-test for parametric values and Mann-Whitney U test for non-parametric values. Differences between three or more compared groups were tested with one-way ANOVA with Scheffé and Newman-Keuls post tests. For nonparametric analysis Kruskal-Wallis ANOVA was used. Friedman ANOVA was used for dependent analysis. The Spearman correlation coefficients were used for correlation analysis. All statistical analyses were performed using versions 7.0 to 9.0 of StatSoft software Statistica (2007, CZ).

4. Results

4.1. Metabolic syndrome

Into the study were included 40 patients with MetS (20 male/20 female) and 40 sex- and age matched volunteers (CON) without MetS. The diagnosis of MetS was done according to the International Diabetes Federation criteria (Alberti, 2005). The activities of CuZnSOD, CAT and PON1-A were assessed. As marker of oxidative stress, concentrations of CD-LDL were determined. Furthermore levels of CuZnSOD cofactors – copper and zinc, PON1 cofactor Ca as well as levels of apo-A1 and HDL-C were determined.

In the MetS group, 21 patients (52.5%) had three, 13 patients (32.5%) four and 6 patients (15.0%) had all five basic components of MetS (abdominal obesity, raised glucose levels, raised TG levels, hypertension, reduced HDL-C).

The subjects with MetS had elevated activities of CuZnSOD ($p < 0.01$) and concentrations of CD-LDL ($p < 0.001$). On the other hand, activities of CAT ($p < 0.05$) and PON1-A ($p < 0.05$) were found to be decreased. Levels of apo-A1 ($p < 0.01$) and HDL-C ($p < 0.001$) were significantly decreased in the patients with MetS. There were found no differences in concentrations of Cu, Zn and Ca between MetS and CON groups.

In the correlation analysis in MetS group, positive correlation was found between PON1 activity and apo-A1 ($r = 0.498$, $p < 0.001$) and HDL-C ($r = 0.459$, $p < 0.01$) concentration. The same correlations were also observed in the whole group (MetS + CON, $N = 80$), furthermore correlations between activity of CuZnSOD and concentration of Zn ($r = 0.363$, $p < 0.01$) were found in the whole group. There were no correlation between CuZnSOD activity and level of serum copper. Negative correlation was observed between activity of CAT and concentration of CD-LDL ($r = -0.233$, $p < 0.05$). The concentration of CD-LDL correlated positively with the number of MetS components ($r = 0.442$, $p < 0.01$).

More results could be found in the publication of Vavrova et al., 2013 (Supplement 1).

4.2. Depressive disorder

In the study of depressive disorder 35 drug-naive women with DD and 35 age-matched healthy women were investigated. Depressive disorder was diagnosed according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, DSM-IV (American Psychiatric Association, 1994). All patients were evaluated using Hamilton Depression Rating Scale (HAM-D). The activities of CuZnSOD, CAT and PON1 – A were observed. As marker of oxidative stress concentrations of CD-LDL were determined. Furthermore levels of CuZnSOD cofactors – copper and zinc, PON1 cofactor Ca as well as levels of apo-A1 and HDL-C were determined.

Erythrocyte activities of CuZnSOD and concentrations of CD in precipitated LDL were increased in depressive women in comparison with healthy CON. Activities of CAT and PON1-A were not altered in patients with DD. No differences were also found in concentrations of copper, zinc, calcium, HDL-C or Apo-A1.

In women with DD, activities of PON1 were positively correlated with concentrations of HDL-C ($r = 0.457$, $p < 0.01$), apo A-1 ($r = 0.379$, $p < 0.05$) and calcium ($r = 0.371$, $p < 0.05$). Furthermore, activities of CuZnSOD were positively correlated with concentrations of zinc ($r = 0.393$, $p < 0.05$), but there were no significant correlation with copper. Positive correlation was observed between activity of CuZnSOD ($r = 0.483$, $p < 0.01$) or CAT ($r = 0.550$, $p < 0.01$) and concentrations of CD-LDL in DD women. In the whole group (DD + CON, $N = 70$) the results were similar; however there were no correlation between activity of CuZnSOD or CAT and levels of CD.

More results could be found in the publication of Kodydkova et al., 2009 (Supplement 3).

4.3. Sepsis and septic shock

Two different studies were done with the patients suffering from sepsis. In the first pilot study only the paraoxonase and arylesterase activities of PON1 and concentrations of HDL-C in serum were determined. Thirty septic patients (SP) and 30 age- and sex-matched outpatient controls (CON) without clinical and laboratory signs of sepsis were included into this pilot study. Patients fulfilled the criteria of sepsis according to the Society of Critical Care Medicine/American College of Chest Physicians

SCCM/ACCP) definitions (Bone, 1992) not longer than 24 hours together with the following criteria for inclusion: APACHE II score ≥ 10 (Knaus, 1985) and C-reactive protein in serum > 20 mg/l. The samples of SP were taken two times during the first 24 hours after onset of sepsis and then 7 days after recovery. The samples after recovery were available in 11 patients. Six patients (20 %) died of sepsis. The main source of sepsis were lungs – in 14 cases, other sources were venous catheter infection (6 cases), abdominal infection (6 cases) or urinary tract infection (3 cases).

Significantly decreased arylesterase and paraoxonase activities of PON1 and levels of HDL-C were found in SP relative to controls. After recovery both activities of PON1 reached nearly the control levels, although a significant increase compared to the onset of sepsis was observed only in activity of PON1-A. Also the levels of HDL-C reached the control level and were significantly higher after recovery in comparison with those in sepsis. No significant difference between survivors and non-survivors was found in the PON1 activities, however there was a trend toward lower arylesterase PON1 activity in non-survivors as compared to survivors (64.94 ± 29.83 vs. 94.13 ± 36.14; $p = 0.07$). Regardless of the source of sepsis, there were no differences in PON1 activities or concentrations of HDL-C among septic patients.

In this study strong positive correlation was demonstrated between both PON1 activities ($r = 0.725$, $p < 0.001$) in the whole group (SP + CON, N = 60) as well as in the SP ($r = 0.667$, $p < 0.001$). Both PON1 activities also correlated positively with the HDL-C concentrations (PON1-A: $r = 0.684$, $p < 0.001$; PON1-P: $r = 0.352$, $p < 0.01$) in the whole group, while in SP there was significantly positive correlation only between PON1-A and HDL-C ($r = 0.560$, $p < 0.01$).

More results could be found in the publication of Novak et al., 2010 (Supplement 4).

Thirty SP, 30 healthy CON and 15 critically ill patients without sepsis (NS) were involved into the second study concerned with sepsis. The criteria of involvement into the study were the same as in the pilot study. The samples of SP were taken three times during the first 24 hours after onset of sepsis (S1), 7 days after the first sampling (S7) and then 7 days after recovery (R7). The samples after recovery were available in 19 patients. Samples from CON and non-septic group were obtained once. Eight patients

(26.7%) died of sepsis and 3 patients were lost from follow up because they never fully recovered from sepsis.

In the first part of this study the changes in antioxidant status and oxidative stress markers were analysed in severe sepsis/septic shock and after the clinical recovery phases and compared with CON. The activities of CuZnSOD, CAT and PON1-A were determined together with levels of oxidative stress markers: CD-LDL, ox-LDL and NT. The concentrations of HDL-C, Apo-A1, Ca, Cu, Zn, Fe and SAA were also measured.

In this study CuZnSOD activity was increased in S1 and returned to the CON value already in S7. The decline in the CAT activity found in S1 and S7, returned to the CON level in R7. Marked fall in the PON1 activity appeared at the onset (S1) and persisted until recovery (R7). Decreased PON1 activity was closely followed by the decrease in HDL-C and ApoA1 concentrations. Whereas SAA concentration was significantly increased in S1, marked decline was observed in S7 and reached nearly CON level in R7. Furthermore the decrease in Zn was observed in both S1 and S7 compared to HC, nevertheless the decline returned nearly to the CON values in R7. However, the decrease in Fe observed in S1, persisted still 7 days after recovery (R7) and never reached the CON levels.

The levels of ox-LDL/LDL, CD/LDL and nitrotyrosine were increased in S1, culminated in S7 and returned to the HC values in R7.

In the septic group (S1) positive correlation was found between PON1 activity and concentration of HDL-C ($r = 0.613$, $p < 0.01$) and negative correlation was observed between PON1 activity and levels of CD/LDL ($r = -0.632$, $p < 0.05$) and ox-LDL/LDL ($r = -0.605$, $p < 0.05$). The levels of CD/LDL correlated positively with levels of ox-LDL/LDL ($r = 0.644$, $p < 0.01$). No significant correlation was found between activity of CuZnSOD and concentrations of Cu or Zn and between CAT and concentration of Fe.

More results could be found in the publication of Vavrova et al. (currently under review; supplement 7).

In the second part of this study activities of antioxidant enzymes and markers of oxidative stress in septic patients and non-septic critically ill patients were compared. As previously mentioned 15 critically ill patients without sepsis were enrolled into the

study according to their sex, age and APACHE II score. These NS patients were matched with SP patients. The activities of CuZnSOD, CAT and PON1-A were determined together with levels of oxidative stress markers: CD-LDL and ox-LDL. The concentrations of HDL-C, Apo-A1, Cu, Zn, Fe and SAA were also measured. The basic characteristics are summarised in Table 2.

Table 2: Basic characteristics of septic patients and non-septic patients

	SP	**NS**	**CON**
N (M/F)	15 (9/6)	15 (9/6)	15 (9/6)
Age (years)	74 (61-79)	70 (57-79)	71 (58-79)
APACHE II score	16 (13-20)	17 (13-20)	-
CRP (mg/l)	96.0 (47.0-185.5)***	84.8 (4.8-130.6)**	2.1 (2.8-7.8)
PCT (mg/l)	2.39 (0.79-10.0)*$^{+++}$	0.28 (0.14-0.73)	0.585 (0.32-0.90)
IL-6 (µg/l)	114.0 (51.0-313.1)***	21.5 (10.9-48)**	1.15 (0.58 - 2.86)
IL-10 (µg/l)	8.58 (5.12 - 16.57)***	5.16 (1.76-6.98)***	0.79 (0.00 - 1.03)
TNF- α(µg/l)	21.8 (11.9 - 39.2)***	6.54 (4.16 - 9.50)	11.89 (6.82 - 14.47)
TC (mmol/l)	3.04 ± 0.71***	3.31 ± 1.14***	5.77 ± 1.05
TG (mmol/l)	1.30 ± 0.48	1.28 ± 0.51	1.41 ± 0.68
LDL-C (mmol/l)	1.76 ± 0.55***	1.77 ± 0.93***	3.67 ± 0.75
Ferritin (µg/l)	336.6 (196.9 - 1297.5)**	356.1 (222.2-1346.8)	278.4 (193.9-646.4)
Transferin (g/l)	1.58 (1.46 -1.91)***	1.92 (1.40 - 2.47)***	2.50 (2.45 - 2.65)

*APACHE II score: Acute Physiology and Chronic Health Evaluation II score, CRP: C-reactive protein, PCT: procalcitonin, IL: interleukin, TNF-α: tumour necrosis factor-α,, LDL: low density lipoprotein, TC: total cholesterol, TG: triglycerides; SP: septic patients, NS: critically ill patients without sepsis, CON: healthy volunteers; *SP or NS vs. CON, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$; $^{+}$SP vs. NS, $^{+++}$ $p < 0.001$, $^{++}$ $p < 0.01$, $^{+}p < 0.05$; one-way ANOVA with Newman-Keuls post test for parametric and Kruskal-Wallis ANOVA for non-parametric analysis.*

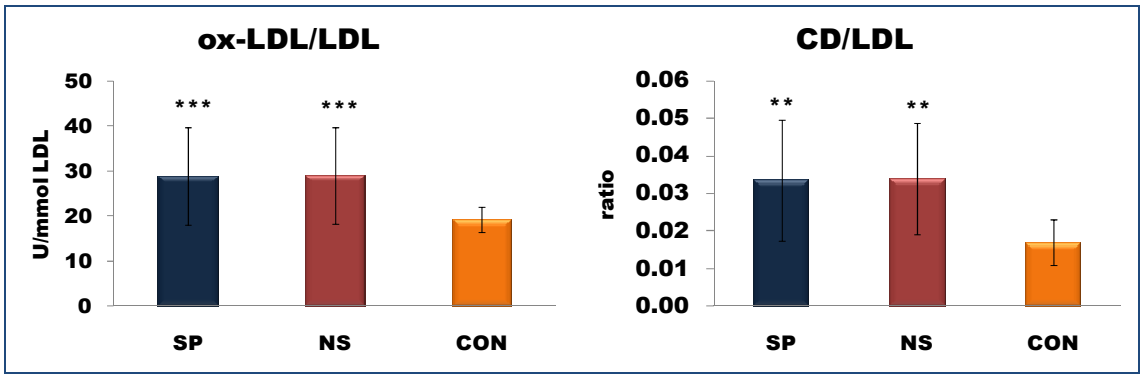


Figure 7: Oxidative stress markers

*SP: septic patients, NS: non-septic critically ill patients, CON: healthy controls; CD: conjugated dienes, ox-LDL: oxidized LDL, LDL: low density lipoprotein; *SP or NS vs. CON, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$; $^{+}$SP vs. NS, $^{++}$ $p < 0.01$, $^{+++}$ $p < 0.001$; one-way ANOVA with Newman-Keuls post test.*

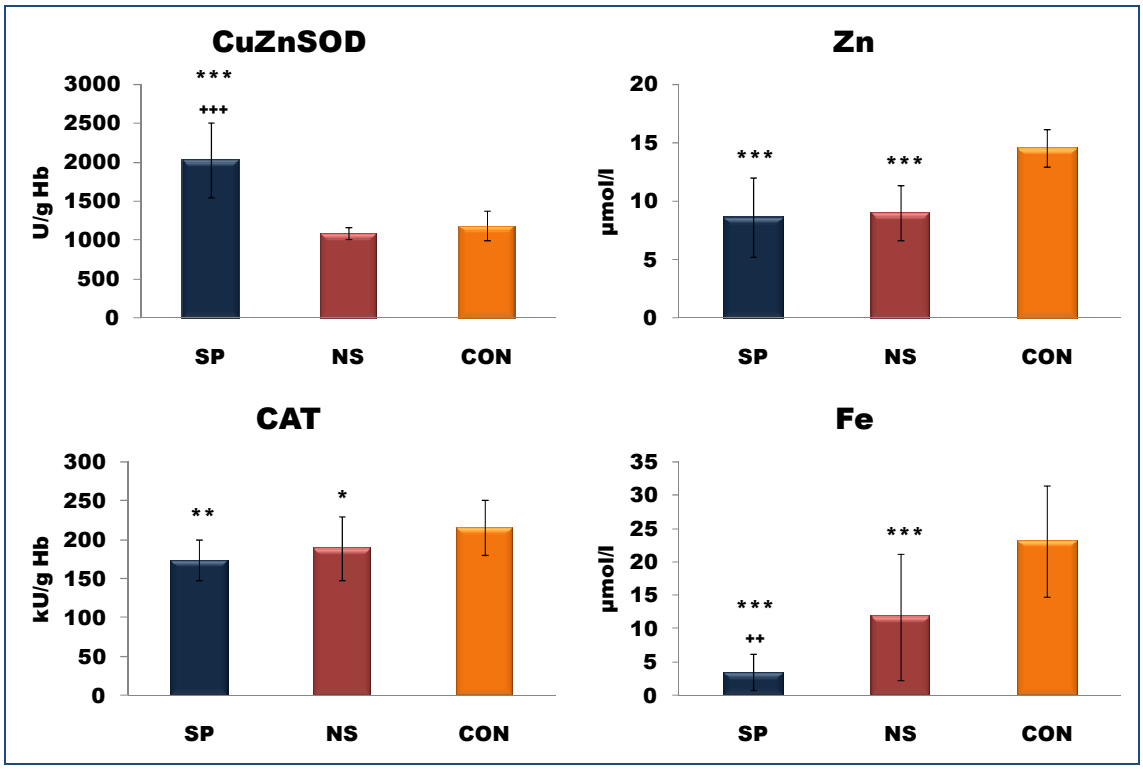


Figure 8: Superoxide dismutase and catalase and their cofactors

*SP: septic patients, NS: non-septic critically ill patients, CON: healthy controls; CuZnSOD: copper-zinc superoxide dismutase, CAT: catalase; *SP or NS vs. CON, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$; $^{+}$SP vs. NS, $^{++}$ $p < 0.01$, $^{+++}$ $p < 0.001$; one-way ANOVA with Newman-Keuls post test.*

As shown in Figure 7 the levels of oxidative stress markers were increased in both patients group (SP and NS, respectively) compared to CON, however there was no difference between the SP and NS subjects.

The activities of CuZnSOD were significantly higher in SP in comparison with NS and with CON. In the contrast to CuZnSOD, activities of CAT and levels of Zn and Fe were significantly decreased in SP as well as in NS compared to CON (Figure 8).

The activities of PON1-A and concentrations of HDL-C and Apo-A1 were depressed in both patient groups compared to CON. In contrast, concentrations of SAA were elevated in SP and NS in comparison with CON (Figure 9).

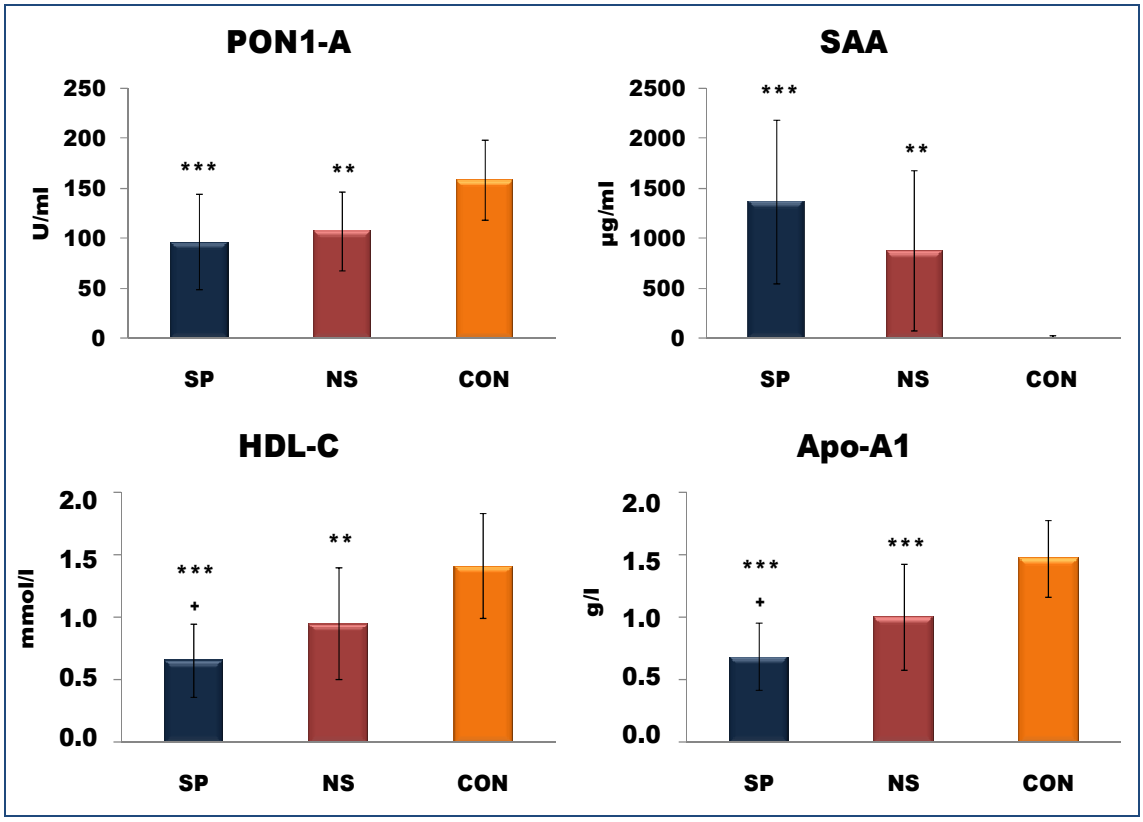


Figure 9: Paraoxonase and parameters connected with PON1 function

*SP: septic patients, NS: non-septic critically ill patients, CON: healthy controls; PON1-A: paraoxonase – arylesterase activity, HDL-C: high density lipoprotein cholesterol, Apo: apolipoprotein, SAA: serum amyloid A; *SP or NS vs. CON, *** $p < 0.0001$, ** $p < 0.001$; $^{+}$SP vs. NS, $^{+}$ $p < 0.05$; one-way ANOVA with Newman-Keuls post test.*

4.4. Acute pancreatitis

Into our study 13 patients with acute pancreatitis (AP) were included together with 13 sex- and age- healthy controls (CON) and 13 sex- and age- matched controls enrolled from persons that suffered from AP 2 – 3 years ago (PAP). In this study, patients in the course of AP were observed. The blood samples were taken four times – in the first 24 hours of disease (AP1), after 72 hours from disease onset (AP3), on the 5^{th} day (AP5) and on the 10^{th} day (AP10) of disease. Gallstones were found as an etiological factor in 8 cases, alcohol intake in 2 cases, endoscopic retrograde cholangio-pancreatography in one case and 2 cases were idiopathic AP. Eight patients suffered from mild form of AP, four patients from moderate and one patient form critical form of AP.

In all studied groups markers of oxidative stress (level of conjugated dienes in precipitated LDL and level of oxidized LDL) and activities of CuZnSOD, CAT, PON1-A and PON1-L were determined. Also the levels of Cu, Zn, Fe, Ca, HDL-C and Apo-A1 were established.

In our study increased oxidative stress in AP was confirmed, with higher levels of CD/LDL in all AP samplings compared to CON ($p < 0.05$) and with increased levels of ox-LDL during the AP with the maximum on the 5^{th} day. Both PON1 activities were depressed in all AP samplings in comparison to CON. The lowest activity of PON1-A was observed on the 5^{th} day. Also the levels of HDL-C and Apo-A1 were decreased in all AP samplings compared to controls. Activities of CuZnSOD and CAT in AP did not differ from those of CON.

Positive correlation between both activities of PON1 ($r = 0.742$, $p < 0.01$) was found in the AP group (AP1). The activity of PON1-A correlated positively with Apo-A1($r = 0.657$, $p < 0.05$) and with concentration of Ca ($r = 0.669$, $p < 0.05$). The activity of PON1-L correlated positively with HDL-C ($r = 0.664$, $p < 0.05$) and also with concentration of Ca ($r = 0.711$, $p < 0.05$). No significant correlation was found between activity of CuZnSOD and concentrations of Cu or Zn and between CAT and concentration of Fe.

More results could be found in the article of Vavrova et al. 2012b (Supplement 5).

4.5. Pancreatic cancer and chronic pancreatitis

The study population consisted of three groups: 50 patients with pancreatic cancer (PC), 50 patients with chronic pancreatitis (CP) and 50 healthy controls (CON). All groups are age and sex matched. Among our 50 patients with PC there were following stage distribution: 9 patients - grade II, 24 patients - grade III, 17 patients - grade IV. Among 50 patients with CP there were 30 patients with severe grade, 17 with moderate, and 3 with mild grade changes in pancreatic morphology. Alcoholic CP was diagnosed in 38 patients, obstructive CP in 5 subjects, and 7 patients experienced idiopathic CP. The activities of CuZnSOD, CAT, PON1 – A and PON1 – L were assessed. As marker of oxidative stress, levels of CD-LDL, Ox-LDL and NT were determined. Furthermore levels of CuZnSOD cofactors – copper and zinc, PON1 cofactor Ca as well as levels of apo-A1, HDL-C and SAA were determined; the concentrations of Fe were also established.

Elevated levels of oxidative stress markers ox-LDL/LDL and CD/LDL were observed in CP and PC compared to CON. The concentrations of NT were found to be increased only in CP in comparison with CON. Increased activities of CuZnSOD and decreased activities of CAT, PON1-A and PON1-L were found in PC compared to CP and CON. The activities of PON1 were depressed also in CP in comparison with CON. In activities of CAT and CuZnSOD no differences between CP and CON were observed. Increased levels of Cu, Zn and SAA and decreased levels of Fe, HDL-C and Apo-A1 were observed in PC relative to CON.

In the whole group (CP + PC + CON, N = 150) negative correlation between activity of CuZnSOD and concentration of Zn ($r = -0.196$, $p < 0.05$) was found. Strong positive correlation between both activities of PON1 ($r = 0.806$; $p < 0.001$) was observed. Both activities of PON1 correlated positively with HDL-C ($r = 0.453$, $p < 0.001$; $r = 0.474$, $p < 0.001$; respectively) and Apo A1 ($r = 0.538$, $p < 0.001$; $r = 0.515$, $p < 0.001$; respectively) and negatively with SAA ($r = -0.278$, $p < 0.01$; $r = -0.384$, $p < 0.001$; respectively). The levels of ox-LDL/LDL correlated positively with concentration of SAA ($r = -0.414$, $p < 0.001$) and negatively with both activities of PON1 ($r = -0.309$, $p < 0.001$; $r = -0.358$, $p < 0.001$; respectively).

More results could be found in the publication of Kodydková et al. 2013 (Supplement 2)

5. Discussion

Activities of three different antioxidant enzymes in erythrocytes: CuZnSOD, CAT and PON1 together with markers of oxidative stress in serum – CD-LDL, ox-LDL and NT were established in six different pathophysiological states. Furthermore serum concentrations of enzymatic cofactors such as Cu, Zn, Fe and Ca and levels of some lipid parameters – HDL-C and apo-A1 and in some studies concentrations of SAA were determined. Patients with metabolic syndrome, depressive disorder, sepsis or septic shock, pancreatic carcinoma, chronic and acute pancreatitis were included into the studies dealing with oxidative stress and status of antioxidant defence system.

In all these above mentioned diseases increased levels of oxidative stress markers were observed compared to CON. In all studies concentrations of CD-LDL were measured. The levels of ox-LDL were determined in patients with CP, PC, AP and sepsis. Although in all studies increased oxidative stress was observed, the activities of measured antioxidant enzymes were not affected in all these diseases.

5.1. Catalase

Decreased activities of catalase were observed in patients with sepsis or septic shock, MetS and PC in comparison with CON, while in patients with DD, CP and AP no differences in CAT activity were detected.

The results of MetS studies previously published are inconsistent. In accordance with our results Koziróg et al. (2010) found decreased CAT activity in erythrocytes of MetS compared to CON. However no difference in erythrocyte CAT activity between MetS and CON were observed in two other studies (Broncel et al., 2010; Pizent et al. 2010). Cardona et al. (2008a, 2008b) found increased activities of serum CAT in MetS. Furthermore decreased activities of CAT were described in patients bearing only individual components of MetS – obesity (Viroonudomphol, 2000), hypertension (Rodrigo, 2007) or insulin resistance (Shin, 2006).

Activities of CAT in erythrocytes were not altered in our set of DD women, in accordance with Bilici et al. (2001). However, Galecki et al. (2009) observed increased activity of CAT in erythrocytes in depressive patients compared to CON, Szuster-

Ciesielska et al. (2008) found raised activities of CAT in serum of patients with major depression. In the study of patients with multiple sclerosis Miller et al. (2011) found elevated erythrocyte CAT activity in comparison with controls, regardless of the depression. Ozcan et al. (2004) described decreased CAT activities in erythrocytes of patients with affective disorders.

In the studies dealing with activities of CAT in SP the results are contradictory to our ones. Increased CAT activity was found in both erythrocytes and plasma (Warner et al., 1995) and also in serum (Leff et al., 1992 and Leff et al., 1993) of adult SP. Increased serum CAT activity was observed also in neonatal sepsis (Kapoor et al., 2006). Leff et al. (1993) had reported that SP suffering from acute respiratory distress syndrome (ARDS) had higher activities of serum CAT than those SP without ARDS. However Metnitz et al. (1999) did not find any alterations in the erythrocyte CAT activity in patients with ARDS.

To the best of my knowledge, there is only one study dealing with the CAT activity in PC and in the contrast to our study, no changes in CAT activity were found (Fukui 2004).

No significant difference in CAT activities of CP patients observed in our study, were consistent with results of Fukui et al. (2004). In the contrast, other authors described increased (Szuster-Ciesielska, 2001a, Szuster-Ciesielska, 2001b) and also decreased (Quillot, 2005) CAT activities in patients with CP.

In accordance with our results no difference in erythrocyte CAT activity between AP and CON were found in study of Chmiel et al. (2002). It was shown, that serum CAT activity was higher in AP than in CON (Goth, 1989; Góth, 1982; Szuster-Czielska, 2001a; Fukui, 2004).

As mentioned above we found either decreased or unchanged activities of erythocyte CAT in observed disaeses. All these diseases were conected with increased oxidative stress as shown with elavated levels of CD-LDL and ox-LDL. Oxidative stress is caused by the imbalance between RONS production and degradation. It could be supposed, that in higher concentration of hydrogen peroxide, also the activity of CAT will be increased. It is known that CAT belongs to the most effective enzymes, the catalactic rate of catalase is among highest of known enzymatic rates (Kirkman et al., 2006).

However it was previously shown that long-term exposure of CAT to H_2O_2 leads to the oxidation of the catalase bound NADPH to $NADP^+$ and to a decrease in the initial activity of CAT about to 30 % of the initial activity (Kirkman et al., 1987).

5.2. Superoxide dismutase

Increased activities of CuZnSOD were observed in MetS, DD, PC and SP, while no difference in CuZnSOD activities were found between CP or AP and CON.

The raised CuZnSOD activities in the erythrocytes of patients with MetS found in our study may be compared with the results of Dimitrijevic-Sreckovic et al. (2007), who described slightly increased CuZnSOD activities in children with MetS in comparison with obese children without MetS. However the previously described results are not consistent, then in some studies decreased CuZnSOD activity in MetS patients were observed (Koziróg et al., 2010; Broncel et al., 2010) and in study of Pizent et al. (2010) no difference were found between MetS patients and CON. No difference between MetS and CON were also observed in EC-SOD activity (Cardona et al., 2008a).

We have found increased CuZnSOD activities in erythrocytes of DD compared with CON, similarly to Sarandol et al. (2007), Bilici et al. (2001), Gałecki et al. (2009) and Kotan et al. (2011). Inconsistent results were published for serum EC-SOD activities. Herken et al. (2007) and Selek et al. (2008) have found decreased, whereas Khanzode et al. (2003) and Szuster-Ciesielska et al. (2008) elevated EC-SOD activities in patients with major depression.

In line with our results of the study with patients with sepsis, Warner et al. (1995) found the increased activity of CuZnSOD at the onset of sepsis. On the other hand no differences in CuZnSOD activity between septic and healthy children (Cherian et al., 2007) as well as in patients with ARDS were detected (Metnitz et al., 1999). Also the activities of EC-SOD were found to be elevated in septic neonates compared to CON (Batra et al., 2000; Kapoor et al., 2006). Mühl et al. (2011) did not find any difference in EC-SOD activity between adult septic patients and CON.

No significant difference in the activities of CuZnSOD in CP patients and controls, found in our study, were consistent with the study of Quillot et.al. (2005). On the other hand, decreased CuZnSOD activity in CP patients was found in the study of Girish et al. (2011). Inconsistent results concerning serum SOD activities in hereditary and alcohol-

related pancreatitis have been published. Some reports have described increased (Mathew et al., 1996) or decreased (Szuster-Ciesielska et al., 2001a) SOD activity and in some studies no difference in SOD activities were found (Quillot et al., 2005, Quilliot et al., 2001).

In the study with AP patients we didn't find any difference between CuZnSOD activity in AP and CON. Furthermore the levels of CuZnSOD were stable in the course of AP. The published results about CuZnSOD activity in AP are inconsistent. Some studies observed increased (Chmiel et al., 2002) and some decreased (Abu-Hilal et al., 2006; Park et al., 2003) CuZnSOD activity in patients with mild and/or severe AP. For EC-SOD, increased levels in AP were described (Góth et al., 1982; Góth, 1989; Szuster-Czielska et al., 2001a; Thareja et al., 2009).

5.3. Paraoxonase

The arylesterase activity of PON1 was measured in all observed pathophysiological states and was found to be decreased in all these situations (with the exception of DD) in comparison with CON. In patients with PC, CP and AP also the lactonase activity of PON1 was determined. In PC, CP and AP patients also PON1-L activity was found to be depressed compared with CON. As for paraoxonase activity of PON1, it was established only in the pilot study with septic patients. In septic patients lower activity of PON1-P then in CON was found. The PON1-P activity was not determined more because paraoxon which is used as substrate belongs to carcinogens.

The finding of decreased PON1-A in our subjects with MetS is in accordance with other studies (Hashemi et al., 2011; Kappelle et al., 2011; Martinelli et al., 2012). In patients with MetS were also found decreased PON1-P activities (Sentí et al., 2003; Garin et al., 2005; Rizos et al., 2005; Park et al., 2010; Hashemi et al., 2011; Akçay et al., 2011; Martinelli et al., 2012). However in studies of Tabur et al. (2010) and Lagos et al. (2009) equivalent levels of PON1-P and PON1-A in MetS patients and in CON were found. Also in study of Yilmaz et al. (2010) no difference in PON1-P activities between MetS and CON were observed, however MetS patients with coronary artery disease (CAD) had significantly lower activity of PON1-P then MetS patients without CAD ($p < 0.008$). No difference in PON1-L activities between MetS and CON were observed (Martinelli et al., 2012).

No difference in PON1-A activities between DD and CON were found in our study in accordance with study of Sarandol et al. (2006). The published results are not consistent then also decreased levels of PON1-A activities in DD subjects were already described (Barim et al., 2009; Kotan et al., 2011). In all studies dealing with PON1-P activity equal levels of PON1-P were observed in DD subjects and CON (Sarandol et al., 2006; Barim et al., 2009; Kotan et al., 2011).

In both our studies, deeling with critically ill SP, we found lower PON1-A activity in in comparison with CON in accordance with the study of Draganov et al. (2010). Also PON1-P activities were found to be decreased in SP compared to CON in our pilot study. Similarly Kedage et al. (2010) observed decreased activity of serum PON1-P in septic patients.

We found decreased PON1-A and PON1-L activities in PC patients compared to CON. At the present time, the decreased PON1 activity in PC patients was described only in one study (Akçay et al., 2003a). However decreased PON1-A and/or PON1-P activities were observed in other malignancies such as in breast cancer (Samra et al., 2011), prostate cancer (Samra et al., 2011), lung cancer (Elkiran et al., 2007; Samra et al., 2011), laryngeal cancer (Karaman et al., 2010), endometrial cancer (Arioz et al., 2009), gastroesophageal cancer (Krzystek-Korpacka et al., 2008), gastric cancer (Akçay et al., 2003b) ovarian cancer (Camuzcuoglu et al., 2009), cervix carcinoma (Samra et al., 2011), lymphoma (Samra et al., 2011), high grade gliomas (Kafadar et al., 2006) or meningiomas (Kafadar et al., 2006). There is no study dealing with PON1-L activities in patients with any type of cancer.

The activity of PON1-A was established in experimental AP on animal model (36 male Wistar rats). The pancreatitis was induced by retrograde infusion into the biliopancreatic duct of 5% sodium taurocholate (severe AP) or of 1% sodium taurocholate (mild AP). Control animals received an intraductal infusion of saline solution (0.9% NaCl). In this study they found no changes in PON1-A activity 3 hours after AP induction, by contrast after 18 hours after AP induction they observed significant decrease in PON1-A activity in severe AP compared to controls (Franco-Pons et al., 2008).

In all studies, where two different activities of PON1 were established, both PON1 activities correlated with each other. PON1-A activities correlated positively with PON1-P activities in septic patients and with PON1-L activities in patients with PC, CP

and AP in our studies. Positive correlations between PON1-P and PON1-A were previously described in patients with MetS (Tabur et al., 2010; Hashemi et al., 2011) or with ovarian cancer (Camuzcuoglu et al., 2009).

Because PON1 is carried in plasma/serum bound to HDL through Apo-A1, the concentrations of HDL and Apo-A1 in serum and correlations between PON1 activities and concentrations of HDL and Apo-A1 were also established. In accordance with the results of PON1-A activity in patients with MetS, sepsis or septic shock, PC and AP decreased concentrations of both HDL and Apo-A1 were found. Positive correlation between PON1-A activity and HDL concentration was found in patients with DD, MetS and sepsis or septic shock. Positive correlation between PON1-A activity and Apo-A1 concentration was observed in patients with DD, MetS, sepsis or septic shock, CP and AP. It could be hypothesize, that changes in composition of HDL influence the activity and function of PON1.

Previously was shown that during the acute phase response, HDL structure is modified. It loses esterified cholesterol, apo-A1, and most of the HDL-associated enzymes including PON1 and that afterwards PON1 is replaced by SAA. These changes lead to the loss of HDL antioxidative properties (Van Leeuwen, 2003). The relationship between SAA concentrations and PON1 activities was also demonstrated in patients with MetS (Kappelle et al., 2011).

The levels of SAA were investigated in our studies with CP, PC and SP. Patients with PC and SP had higher concentrations of SAA than CON, while the SAA levels in CP were equal to those of CON. In these studied groups no correlations between PON1 activities and levels of SAA were observed. The finding of increased SAA levels in PC patients in our study is consistent with results of other studies (Yokoi et al., 2005; Firpo et al., 2009). SAA was associated with tumour progression and its metastasizing (Malle et al., 2009). Some authors considered SAA as a tumour marker for PC, however, SAA did not reach appropriate specificity and sensitivity for PC diagnostics (Yokoi et al., 2005; Firpo et al., 2009). Elevated levels of SAA in sepsis were also demonstrated previously (Eras et al, 2011; Cetinkaya et al., 2009; Arnon et al., 2007).

Severeal mechanisms are supposed to decrease PON1 activity. It was shown, that increased oxidative stress connected with elevated levels of oxidized LDL cause inactivation of PON1. Oxidized LDL appears to inactivate PON1 through interactions

between the enzyme's free sulfhydryl group and oxidized lipids, which are formed during LDL oxidation (Aviram et al., 1999). Hydroperoxides of LA inhibit the PON1 activity through the reaction with sulfhydryl group of its cystein 284 (Tavori et al., 2011). Other reason for the decrease in PON1 activity could be the glycation of the enzyme, which takes place as was shown in diabetes mellitus (Hedrick et al., 2000). The acute phase response could also lead to the decreased activities of PON1 which are caused by the down-regulation of liver PON1 mRNA (Deakin & James, 2004). In the model of experimental acute pancreatitis was shown that the decrease of PON1 activities is connected with its inhibition by oxidized lipids and higher proteolytic degradation (Franco-Pons et al., 2008).

6. Conclusions

This doctoral thesis was focused on the behaviour of the antioxidant enzymes – catalase, superoxide dismutase and paraoxonase in different pathophysiological states. Activities of these enzymes were investigated in patients with metabolic syndrome, depressive disorder, sepsis, pancreatic cancer, chronic, and acute pancreatitis.

In patients with metabolic syndrome activities of all three enzymes were altered in comparison with healthy controls. The erythrocyte activities of CuZnSOD were elevated and activities of CAT in erythrocytes and PON1-A in serum were decreased in the MetS patients.

In women with depressive disorders only erythrocyte activities of CuZnSOD were increased compared to controls. Activities of CAT and PON1-A were not altered in DD women.

Patients with sepsis had elevated levels of CuZnSOD activities and decreased activities of CAT and PON1-A and PON1-P compared to controls.

Patients with pancreatic carcinoma had elevated erythrocyte activities of CuZnSOD and decreased activities of CAT, PON1-A and PON1-L in comparison with controls. In patients with acute and chronic pancreatitis only activities of PON1 were depressed compared to controls. In activities of CAT and CuZnSOD no difference between AP or CP and CON were observed.

It was also shown that the different types of paraoxonase activities correlate with each other.

Our studies show that in pathophysiological states the activity of CuZnSOD is elevated and activities of catalase and paraoxonase are depressed when changed.

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8. Publications

A. With IF

1) **Vávrová L**, Kodydková J, Zeman M, Dušejovská M, Macášek J, Staňková B, Tvrzická E, Žák A.: *Altered Activities of Antioxidant Enzymes in Patients with Metabolic Syndrome.* Obesity Facts. 2013;6(1):39-47. (**IF: 1.856**, 2011)
2) Kodydková J, **Vávrová L**, Staňková B, Macášek J, Krechler T, Žák A.: *Changes in antioxidants and oxidative stress markers in pancreatic diseases.* Pancreas. 2013; 42(4):614-21. (**IF: 2.386**, 2011).
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B. Without IF

1) **Vávrová L**, Kodydková J, Macášek J, Ulrych J, Žák A.: *Oxidační stres v průběhu akutní pankreatitidy.* Klin biochem metab. 2012; 20(41): 188-193.
2) Kocík M, Zimovjanová M, Petruželka L, Kodydková J, **Vávrová L**, Žák A.: Oxidative stress after anthracycline therapy in patients with solid tumors. Cas Lek Cesk. 2012;151(10):463-7.
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C. Under review

1) **Vávrová L**, Kodydková J, Mráčkova M, Novák F, sr., Nováková O, Žák A, Novák F.: Changes in antioxidant status and inflammatory response markers persist long after clinical recovery from severe sepsis. 2012

9. Abstracts on the topics of the doctoral thesis

1) Kodydková J., **Vávrová L.**, Staňková B., Macášek J., Krechler T., Žák A.: *Změny aktivit antioxidačních enzymů a markerů oxiadčního stresu u pacientů s karcinomem pankreatu a chronickou pankreatitidou.* Konference Šobrův den, 6. 6. 2012
2) Žák A, Vecka M, Kodydková J, **Vávrová L**, Macášek J, Krechler T, Muravská A, Jáchymová M.: *Polymorfismy a aktivity paraoxonasy u karcinomu pankreatu.* Konference Šobrův den, 6. 6. 2012
3) Kodydková J, **Vávrová L**, Macášek J, Krechler T, Žák A.: *Antioxidační enzymy a karcinom pankreatu.* Cas Lek Cesk, 2012; 151(1).
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5) Kodydková J, **Vávrová L**, Macášek J, Krechler T, Žák A.: *Antioxidační enzymy a karcinom pankreatu.* Sborník Atherosklerosa 2011, ISBN: 978-80-254-8809-6, S.: 58-61.
6) Kodydková J, **Vávrová L**, Krechler T, Žák A.: *Changes in antioxidant enzymes activities in patients with carcinoma of pancreas.* European Journal of Internal Medicine, 2011, 22 (S1): S48-S49. (IF = 1.657)
7) **Vávrová L**, Kodydková J, Macášek J, Krechler T, Žák A.: *Antioxidant enzymes activities in pancretic carcinoma and the influence of diabetes mellitus.* European Journal of Internal Medicine, 2011, 22(S1): S98. (IF = 1.657)
8) **Vávrová L**, Kodydková J, Zeman M, Tvrzická E, Žák A: *Aktivity antioxidačních enzymů u pacientů s metabolickým syndromem.* Sborník abstraktů přednášek – Diagnostika, léčba a prevence závažných civilizačních onemocnění. 2010, ISBN: 978-80-7177-034-3, S.: 58.
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20) Kodydková J, **Vávrová L**, Tvrzická E, Zeman M, Novák F. jr., Žák A.: Paraoxonasa – *Patofysiologické a klinické aspekty.* Sborník Atherosklerosa 2007, ISBN: 978-80-254-0238-2, S.: S19-S27.

21) **Vávrová L**, Kodydková J, Novák F, Novák F. jr., Žák A.: *Snížená aktivita katalasy u pacientů v sepsi.* Sborník 8. studentská konference 1. LF UK, Praha 2007:49.

22) **Vávrová L**, Kodydková J, Novák F jr., Nováková O, Novák F.: *Decreased Catalase Activity in Septic Patients.* Sborník abstrakt, Second Central & Eastern Europe Conference on Health and the Environment, SR, Bratislava, Oct 22-25, 2006, 47.

10. Supplements

Supplement 1:

Vávrová L, Kodydková J, Zeman M, Dušejovská M, Macášek J, Staňková B, Tvrzická E, Žák A.: *Altered Activities of Antioxidant Enzymes in Patients with Metabolic Syndrome.* Obesity Facts. 2013;6(1):39-47. (**IF: 1.856**, 2011)

Supplement 2:

Kodydková J, **Vávrová L**, Staňková B, Macášek J, Krechler T, Žák A.: *Changes in antioxidants and oxidative stress markers in pancreatic diseases.* Pancreas. 2013; 42(4):614-21. (**IF: 2.386**, 2011)

Supplement 3:

Kodydková J, Vávrová L, Zeman M, Jirák R, Macášek J, Staňková B, Tvrzická E, Žák A.: *Antioxidative enzymes and increased oxidative stress in depressive women.* Clinical Biochemistry 2009; 42: 1368-74. (**IF: 2.019**)

Supplement 4:

Novák F, Vávrová L, Kodydková J, Novák F Sr, Hynková M, Žák A, Nováková O.: *Decreased paraoxonase activity in critically ill patients with sepsis.* Clin Exp Med 2010; 10: 21-25. (**IF: 1.6**)

Supplement 5:

Vávrová L, Kodydková J, Macášek J, Ulrych J, Žák A.: *Oxidační stres v průběhu akutní pankreatitidy.* Klin biochem metab 2012; 20(41): 188-193. (**IF: 0.0**)

Supplement 6:

Macášek J, Zeman M, Vecka M, Vávrová L, Kodydková J, Tvrzická E, Žák A.: *Reactive oxygen and nitrogen species in the clinical medicine.* Cas Lek Cesk 2011; 150(8): 423-32. (**IF: 0.0**)

Supplement 7:

Vávrová L, Kodydková J, Mráčkova M, Novák F, sr., Nováková O, Žák A, Novák F.: *Increased inflammatory cytokines together with impaired antioxidant status persist long after clinical recovery from severe sepsis: correlation with HDL-cholesterol and albumin.* 2013 (under review).